CUK-H03737-55-P024743

VOL-110
NO. 7-9
2001

VOLUME 110 — JULY 2001
umber 7, Part 1 (Issued in 2 Parts)

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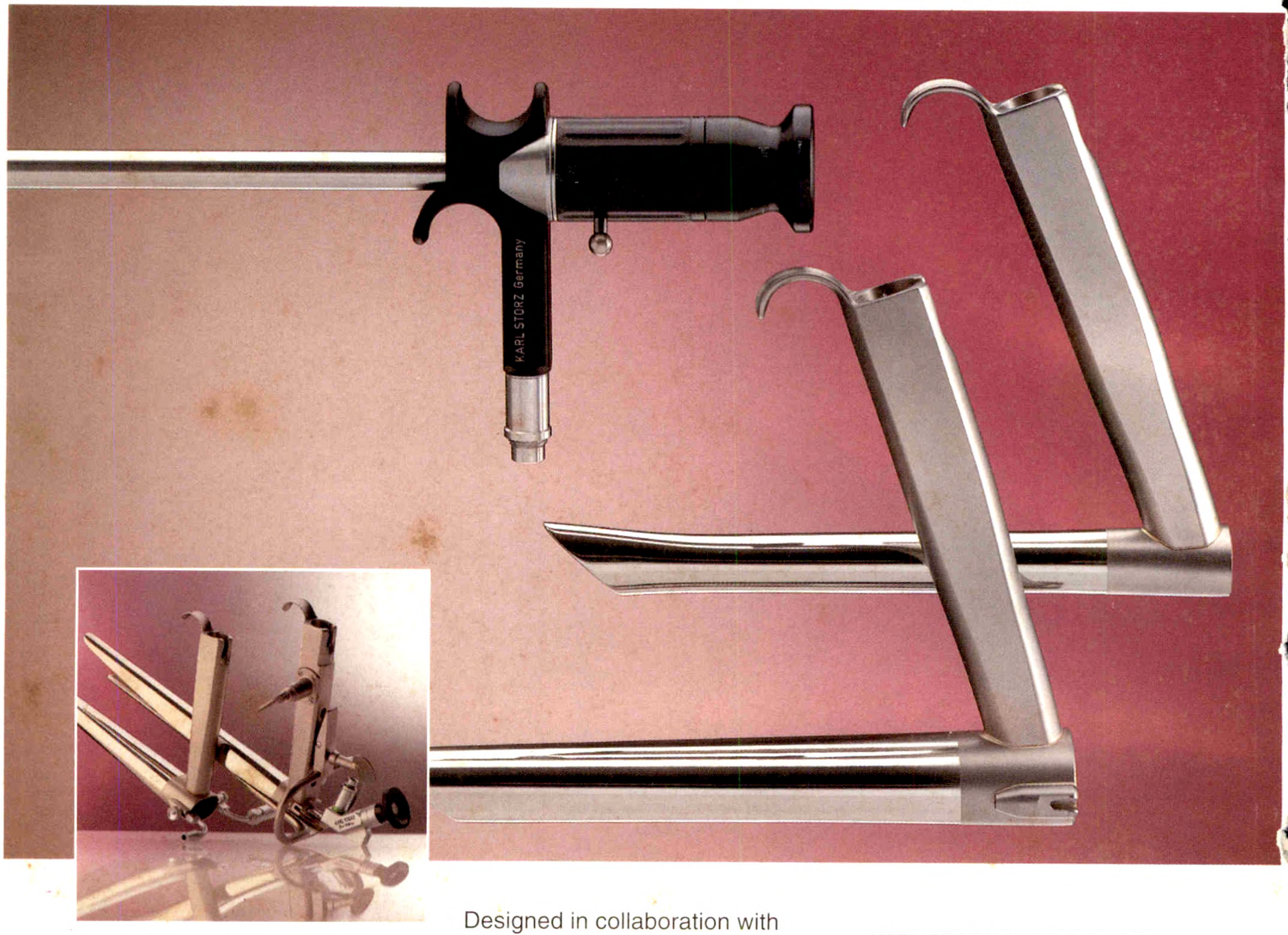

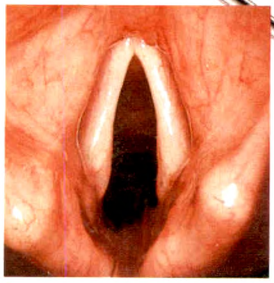

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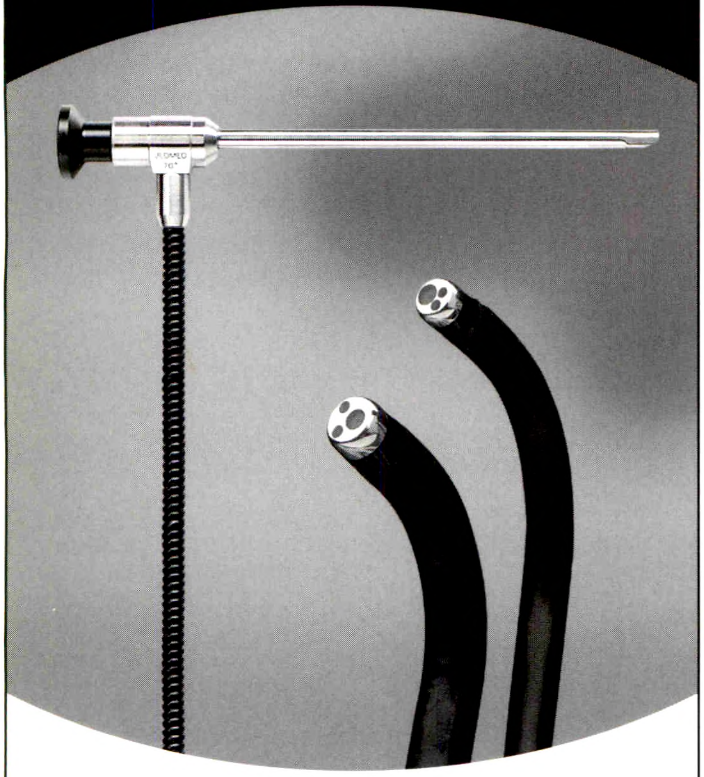

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CRITERIA FOR AUTHORSHIP

In 1985, the International Committee of Medical Journal Editors (also known as the Vancouver Group) drew up a set of criteria for authorship to be incorporated into their *Uniform Requirements for Manuscripts Submitted to Biomedical Journals.*[1] The Committee members took this step partly to discourage the inflationary increase they had noticed in the number of authors listed on biomedical papers and partly to put an end, they hoped, to the practice of "gift" authorship. Discussion of the responsibility and integrity of authorship has been a heated topic of late in otolaryngology journals.[2-4] The *Annals*, too, has noted a steady increase in the number of individuals listed as "authors" on manuscripts submitted for peer review. Further, an alarming increase in requests for changes in authorship *after* the acceptance of a manuscript has also been observed. We now join our efforts with those of other prominent journals in our field in defining who may or may not be considered an author on articles submitted, in restricting the number of authors on articles, and in ensuring the integrity of the work published.

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DIAGNOSIS OF RECURRENT INTERMITTENT AIRWAY OBSTRUCTION ("RECURRENT CROUP") IN CHILDREN

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Children with a history of recurrent intermittent airway obstruction can present with a constellation of signs and symptoms labeled by their primary care physician as "recurrent croup." Recurrent croup, however, *is not a diagnosis* and should alert the clinician to the potential for intrinsic or extrinsic laryngotracheal pathology. This study was undertaken to evaluate referral patterns, diagnostic algorithms, and diagnoses for children with a history of recurrent intermittent airway obstruction initially characterized as "recurrent croup." We performed a retrospective analysis of office and operative records of children with a history of recurrent intermittent airway obstruction characterized as "recurrent croup." The setting was a university-based pediatric otolaryngology practice with both urban and suburban referral patterns. The medical records of 53 children (median age, 2 years) with a history of mild to severe recurrent intermittent airway obstruction characterized as "recurrent croup" were evaluated with respect to referral source, duration of signs and symptoms, diagnostic algorithm, and findings. We conclude that recurrent intermittent airway obstruction in children is an underappreciated clinical scenario. Upper aerodigestive tract endoscopy is a key component in a diagnostic evaluation to ascertain the cause. The decision for an endoscopic evaluation is best determined after analysis of the patient's age, past medical history, clinical severity, and suspected diagnosis. By virtue of his or her knowledge of aerodigestive tract anatomy and endoscopy skills, the otolaryngologist can take a proactive role in arriving at an accurate diagnosis in these children.

KEY WORDS — airway obstruction, croup, laryngotracheal pathology.

INTRODUCTION

Recurrent intermittent airway obstruction is an atypical clinical pattern in children. The constellation of signs and symptoms may be labeled as "recurrent croup," but this does not represent a specific diagnosis. Croup, or laryngotracheobronchitis, is a relatively common viral inflammatory disorder of the upper conductive airway in children whose typical clinical presentation and diagnosis usually does not involve the otolaryngologist.[1-3] A pattern of "recurrent croup," however, should alert the clinician to consider the potential for other laryngotracheal or mediastinal disease that can have an impact upon airway caliber and/or function.[4] The objective of this study was to evaluate referral patterns, diagnostic algorithms, and diagnoses for children with a history of recurrent intermittent airway obstruction initially characterized as "recurrent croup."

MATERIALS AND METHODS

We reviewed the medical records of 53 children who presented to a pediatric otolaryngology practice between 1994 and 1999 with a history of intermittent airway obstruction characterized as "recurrent croup" by the primary care physician or parent. The patients were initially evaluated in either urban or suburban office settings, unless seen while hospitalized. The referral source, past medical history (including duration, frequency, and severity of signs and symptoms), and family history were recorded. All endoscopic evaluations were performed at the Medical College of Virginia Hospital, a tertiary care university hospital with complete pediatric subspecialty services available. The findings of endoscopic and nonendoscopic diagnostic evaluations were documented.

Clinical severity was subjectively assessed as mild, moderate, or severe for the purpose of this report in the following manner. By definition, no case in a child more than 4 years of age was categorized as mild. If 2 or fewer episodes occurred in 1 calendar year and intervention was adequately managed in an outpatient office setting, cases were categorized as mild. If 3 or more episodes occurred in 1 calendar year, or 2 or more episodes occurred in at least 2 consecutive years, cases were categorized as moderate. A calendar year was defined as the 12-month period from September 1 to August 31. If multiple episodes in 1 or more years required emergent intervention for airway obstruction, but the child was not hospitalized as an inpatient, the case was categorized as moderate. All cases requiring inpatient management, with or without the need for intubation, were categorized as severe.

The office and operative upper aerodigestive tract endoscopy findings were correlated with the child's

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Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

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TABLE 1. DIAGNOSES OF 53 CHILDREN WITH RECURRENT INTERMITTENT AIRWAY OBSTRUCTION EVALUATED AT MEDICAL COLLEGE OF VIRGINIA FROM 1994 TO 1999

Gastroesophageal reflux	10		
Atopic disease	10		
Intrinsic laryngotracheal disease	28		
Acquired laryngotracheal stenosis		14	
Congenital cricotracheal abnormality		9	
Cricoid deformity			2
Fused tracheal rings			1
Tracheal stenosis			6
Genetic exfoliating mucosal disease		2	
Tumor (subglottic hemangioma)		1	
Tracheal foreign body		1	
Primary tracheobronchomalacia		1	
Extrinsic laryngotracheal disease	9		
Innominate artery compression		4	
Congenital cardiac abnormality		3	
Double aortic arch			2
Aberrant ductus arteriosus			1
Mediastinal mass		2	
Esophageal foreign body granuloma			1
Lymphoma			1
Psychogenic cough	1		
Total	58*		

*Total patients (53) less than sum of rows (58) because of 5 patients with multiple diagnoses (see text).

preoperative history. Findings consistent with gastroesophageal reflux disease were laryngeal introitus erythema or edema, hypertrophic-appearing hypopharyngeal mucosa, tracheal mucosal studding, and visualized active reflux during endoscopy. All esophageal mucosal biopsy specimens were independently evaluated by the department of pathology and were considered positive for reflux disease when characteristic chronic inflammatory findings were identified. Endoscopic findings consistent with reactive airway disease were marked intertracheal ring vascularity, tracheal mucosal studding, or the presence of thickened tracheal secretions in the absence of an active respiratory tract infection. A family history of atopic disease, the preoperative patient history, and nasal endoscopic, nasopharyngeal, and oropharyngeal findings were used to help discriminate between reactive airway disease and gastroesophageal reflux aspiration when tracheal mucosal studding was identified.

RESULTS

We reviewed the medical records of 53 children identified with a history of recurrent intermittent upper airway obstruction. Their diagnoses are presented in Table 1. The age range at initial presentation was 6 months to 11 years (median, 2 years). There were 40 boys and 13 girls. The symptoms ranged from "croupy cough" with every upper respiratory tract infection to severe "croup-like" cough more than 2 times per year. The overall time frame of symptoms ranged from 6 weeks to "all their life," with the duration of specific episodes ranging from 24 hours to 6 weeks. "Recurrent croup" histories were categorized as mild in 18 children, moderate in 29 children, and severe in 6 children.

A specific referral for "recurrent croup" from the primary care physician was identified in 4 children. One of these children received simultaneous inpatient consultations for pediatric otolaryngology and pediatric cardiology with a presumptive diagnosis of a congenital cardiovascular anomaly based on the history, barium esophagram, and mediastinal magnetic resonance imaging study. Three children were referred for an "airway evaluation" by pediatric anesthesia because of endotracheal tube size incompatibility with age. Two children were referred by departments of pediatric critical care and pediatric pulmonary medicine. Parents initiated an otolaryngology referral to evaluate "abnormal breathing" or coughing in 5 cases. These 14 children all underwent aerodigestive tract endoscopy under anesthesia. In the other 39 children, a history of recurrent intermittent airway obstruction characterized as "recurrent croup" by the parents was first identified at the initial otolaryngology history-taking session, and 34 children received a recommendation for endoscopy under anesthesia. One set of parents whose child's history was most compatible with reactive airway disease elected to continue an observational management plan without endoscopy.

All but 4 children in this series received their first otolaryngology evaluation in the office. Two children were first seen in the pediatric intensive care unit: 1 subsequently received a diagnosis of primary distal tracheobronchomalacia, and the other of laryngotracheal stenosis. One child was first evaluated in the emergency department. He was subsequently judged to have mediastinal lymphoma. One of 2 children with a congenital cardiovascular anomaly was first evaluated on the general pediatric floor.

Of the 53 children, 47 underwent aerodigestive tract endoscopy under general anesthesia. Four children with histories and office physical examination findings most consistent with reactive airway disease, whose "recurrent croup" histories were categorized as mild, did not undergo endoscopy. One child with severe gastroesophageal reflux, with a croup history categorized as "severe," but scheduled for Nissen fundoplication, did not undergo endoscopy. One child with tracheal deviation and a mediastinal mass seen on chest radiography, in the moderate category, did not undergo endoscopy, as a diagnosis of

TABLE 2. DIFFERENTIAL DIAGNOSIS OF "RECURRENT CROUP" IN CHILDREN

- Inflammatory
  - Allergy
  - Asthma
  - Gastroesophageal reflux
  - Tobacco smoke
  - Environmental irritants
- Congenital
  - Congenital subglottic stenosis
  - Congenital cardiovascular abnormality
  - Tracheoesophageal fistula
  - Laryngotracheoesophageal cleft
  - Tracheobronchomalacia
  - Vocal fold paresis or paralysis
  - Esophageal duplication
  - Congenital cricoid deformity
  - Complete tracheal rings
  - Congenital tracheal stenosis
  - Tracheocele
  - Congenital goiter
- Infectious
  - Viral
  - Para-esophageal abscess
- Traumatic
  - Acquired subglottic stenosis
  - Subglottic cyst
  - Airway foreign body
  - Esophageal foreign body
  - Vocal fold paresis or paralysis
  - Thermal inhalation injury
- Tumor
  - Subglottic hemangioma
  - Lymphangioma
  - Aberrant thyroid tissue
  - Thyroid neoplasm
  - Recurrent respiratory papillomatosis
  - Teratoma (cervical or mediastinal)
  - Thymoma
  - Lymphoma (mediastinal)
  - Neurofibroma or blastoma
  - Mediastinal granuloma
- Miscellaneous
  - Psychogenic
  - Telescoped tracheal rings

mediastinal lymphoma was subsequently obtained and symptoms improved with therapy.

At endoscopy, 9 children had laryngotracheal gastroesophageal reflux findings. Upper esophageal biopsy specimens were obtained in 3 children, of which 2 were positive for chronic inflammation. Six children had findings consistent with reactive airway disease. Eight children had extrinsic tracheal compression. Their diagnoses included innominate artery compression (n = 4), mediastinal granuloma (n = 1), and congenital cardiovascular anomalies (n = 3: 2 with double aortic arches, 1 with an aberrant ductus arteriosus associated with tetralogy of Fallot). Fourteen children with neonatal intubation histories had acquired laryngotracheal stenosis: 1 grade II, and 13 grade I. Thirteen children had congenital intrinsic laryngotracheal disease, 9 of whom had cricotracheal abnormalities. Their diagnoses included tracheal stenosis (n = 6), cricoid deformity (n = 2), and fused tracheal rings (n = 1). The remaining 4 children with congenital intrinsic disease had thickened tracheal secretions associated with exfoliating epidermal disease (n = 2), subglottic hemangioma (n = 1), or primary distal tracheobronchomalacia (n = 1). One child had a retained tracheal foreign body. In the absence of abnormal endoscopy findings other than reversible paradoxical vocal fold mobility, 1 child was judged to have psychogenic cough. Co-morbid reactive airway disease or gastroesophageal reflux findings were noted in 5 children with intrinsic or extrinsic laryngotracheal disease.

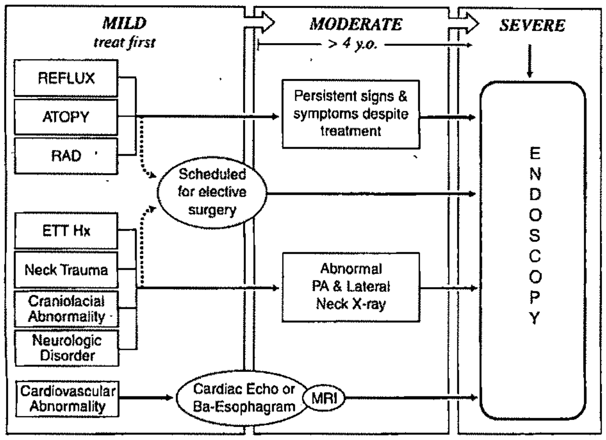
DISCUSSION

BACKGROUND

The underlying cause of recurrent intermittent airway obstruction in children is not always clear at the outset. Infectious laryngotracheobronchitis, commonly referred to as croup, is a well-recognized viral-induced inflammation of the larynx and tracheobronchial tree in children, resulting in a low-grade fever, hoarseness, respiratory difficulty, and a characteristic "barky" cough.[1-3] Unless the patient is infected by an immunologically different strain, it is not typical to have more than 1 episode per year. The otolaryngologist's expertise may be requested when a significant recurrent pattern is identified, prolonged "croup-like" signs persist, or an individual episode results in moderate to severe airway compromise. It is important to recognize that other conductive airway–related disorders can affect the caliber and/or function of the glottis, subglottis, or trachea and may predispose a child to some of the signs and symptoms consistent with croup.[4] "Recurrent croup," however, is not a diagnosis, and particularly in these cases, it is important to assess for other primary or contributing nonviral causes (Table 2).

INITIAL EVALUATION

The evaluation of a child with "recurrent croup" begins with a thorough history. Some parents may bring complete medical records with them, while others are significantly underinformed or unaware of the potential clinical significance. It is important to perform a comprehensive assessment of the signs and symptoms, including the onset, timing, duration, and severity of respiratory distress, past therapeutic interventions and responses, and, not uncommonly, previous evaluations by other specialists. Review of the


MILD
treat first
MODERATE
> 4 y.o.
SEVERE
REFLUX
ATOPY
RAD
Persistent signs & symptoms despite treatment
Scheduled for elective surgery
ETT Hx
Neck Trauma
Craniofacial Abnormality
Neurologic Disorder
Abnormal PA & Lateral Neck X-ray
Cardiovascular Abnormality
Cardiac Echo or Ba-Esophagram
MRI
ENDOSCOPY

Suggested diagnostic algorithm. RAD — reactive airway disease; y.o. — years old; ETT Hx — history of endotracheal tube intubation; PA — posteroanterior; Ba — barium; MRI — magnetic resonance imaging.

neonatal and past surgical histories for relevant cardiac surgery or prior endotracheal intubations can be productive. Prior or ongoing evaluation and management of gastroesophageal reflux or reactive airway disease, as well as their level of clinical control, should be clearly ascertained. Documentation of IgE-mediated disease or a strong family history consistent with atopic disease may be reported. Discussion with the primary care physician and previously consulted specialists in these cases can be helpful and is strongly recommended. The possibility of an ingested or aspirated foreign body must also be considered.

The initial physical examination should be thorough, though it is necessarily limited if the degree of respiratory difficulty demands a more appropriate setting. Signs of chronic obstruction or impending respiratory collapse should be ruled out. The position of the larynx and trachea is noted. Bimanual cervical palpation, cervical auscultation, and assessment for transmitted mediastinal vibrations should be performed. Chest radiographs, fluoroscopic airway evaluations, and high-kilovolt ("soft tissue") radiographic studies may be helpful. We routinely request anteroposterior and lateral cervical radiographs as our first imaging study of choice. Flexible fiberoptic laryngoscopy, in selected patients, can be relatively safely performed^5 and provides useful screening information.

DIAGNOSTIC ALGORITHM

For the purposes of this study, the croup histories were subjectively separated into mild, moderate, and severe categories. By definition, no case in a child more than 4 years of age was categorized as mild, as it was thought that at this age, the expected airway luminal caliber should be great enough to tolerate mild inflammatory responses. A calendar year designed to coincide with the onset of the fall infectious season was used and defined as being from September 1 to the following August 31. Mild recurrent croup was defined as 2 or fewer episodes, adequately managed in the outpatient office setting, occurring in 1 calendar year. Moderate recurrent croup was defined as 1) 3 or more episodes occurring in 1 calendar year, 2) 2 or more episodes occurring in at least 2 consecutive years, or 3) multiple episodes in 1 or more years that required emergent outpatient intervention for airway obstruction. All cases requiring inpatient management, with or without the need for intubation, were defined as severe recurrent croup.

On the basis of this case series, we suggest the diagnostic algorithm in the Figure. We recommend that all children with a history of moderate or severe recurrent airway obstruction undergo aerodigestive tract endoscopy, preferably when clinically quiescent. If the "recurrent croup" history falls into the mild category and, on the basis of the office examination, the most likely cause is thought to be reactive airway disease or gastroesophageal reflux, we recommend considering a medication trial. A failure to significantly improve despite medical intervention automatically advances the case into the moderate category. Alternatively, if a child has a history of mild "recurrent croup" but there is also a history of neonatal intubation, neck trauma, craniofacial abnormality, or neurologic disorder, we recommend obtaining "soft tissue" cervical radiographs in addition to performing an office fiberoptic evaluation. Even if the laryngoscopy findings are normal, if it is determined that there is radiologic evidence of subglottic or tracheal abnormality, such as prolongation of the subglottic interval, the case is automatically advanced into the moderate category.

We recommend elective endoscopy in all children in whom anesthesiologists have encountered difficulty with intubation, despite adequate access to the laryngeal introitus, using an expected normal size-for-age endotracheal tube. Additionally, if a child's "recurrent croup" history falls into the mild category, but he or she has appropriate indications for a surgical procedure under anesthesia, we recommend adding aerodigestive tract endoscopy to the scheduled procedure. More often than not, these children are scheduled for general pediatric otolaryngology procedures such as myringotomy and tube placement or tonsil and/or adenoid surgery. The one major exception to recommending endoscopy as the initial diagnostic procedure is the case in which the child is strongly believed to have a congenital cardiovascular abnormality. In these cases, in addition to plain "soft tissue" cervical radiographs, we recommend a barium esophagram as the initial diagnostic study, with endoscopy performed before intubation at the time of surgical repair. We leave the decision as to whether to obtain a preoperative magnetic resonance imaging study to the primary care physician, pediatric cardiologist, or pediatric cardiac surgeon.

Increasingly sophisticated diagnostic imaging studies have not obviated the need for a thorough endoscopic examination.[6] In this series, the endoscopy findings usually determined whether an esophagram, mediastinal imaging study, or cardiac sonography was necessary. We recognize that the diagnosis of 1 of the 2 cases of double aortic arch anomalies in this series was not made until endoscopy. Certainly, however, some cases of "recurrent croup" present with a presumed diagnosis based on previous surgery or an existing diagnostic workup. It is important to remember that technological advances are always forthcoming and that it is incumbent, therefore, upon the bronchoesophagologist to remain aware of their capabilities. Supported by this series, we maintain that there is still no substitute for direct visualization and that consideration for endoscopy in these children is warranted to document the baseline findings.[7]

ENDOSCOPY

A review of the potential differential diagnosis establishes the need to be familiar with the embryology and development of both the aerodigestive tract and the cardiovascular system. One needs, further, to be familiar with the various tumors that can develop within or around the upper conductive airway, as well as with the ways that the airway can be internally traumatized. The endoscopist must be thoroughly familiar with the normal proportions, dimensions, and appearance of the pediatric aerodigestive tract at various ages. Our preferred diagnostic endoscopy technique is flexible and rigid direct laryngoscopy and rigid tracheobronchoscopy under insufflation anesthesia with spontaneous ventilations in order to evaluate the airway under dynamic conditions. Complementary use of open tube bronchoscopy may be used as indicated by the clinical presentation and findings. If laryngoscopy and tracheobronchoscopy is not sufficiently diagnostic, esophagoscopy with upper esophageal mucosal biopsy is performed.

DIAGNOSIS

The interpretation of aerodigestive tract endoscopy findings is necessarily subjective and subject to the experience of the observer. We recognize that "reactive airway disease" and "reflux" can have different clinical definitions among various clinicians. In our interpretations of the endoscopic findings, we have tried to be faithful to the teachings of our bronchoesophagology heritage, as well as our own clinical experience. All esophageal mucosa specimens were obtained from the posterior aspect of the superior segment. The pathologists evaluated for the presence of lymphocytic infiltrate (eosinophils), basal cell layer hyperplasia, and papillary elongation. We recognize that a specific diagnosis of atopic disease needs to be supported by a strong family history or documentation of IgE-mediated disease. Although requested as part of the patient history, we elected not to include detailed historical data regarding prior diagnostic evaluation or medication use for reactive airway disease or reflux, as this case series was not designed to control for the variability in diagnostic acumen and therapeutic protocols among our regional primary care specialists.

MANAGEMENT

After endoscopy, many patients in this study were given clinical trials of antireflux, antibronchospastic, or anti-inflammatory medications under primary care physician guidance. Alternatively, in several cases, the primary care physician elected to recommend additional testing for gastroesophageal reflux, allergy, or asthma. Patients with a resectable soft tissue or fixed laryngotracheal disorder, such as acquired subglottic cysts or grade II subglottic stenosis, subsequently underwent surgical intervention. In the cases of mild fixed laryngotracheal disorders, such as mild congenital tracheal stenosis, a recommendation to receive inhaled steroid therapy at the onset of respiratory tract infections was usually given. Surgical or medical intervention for noncardiac tracheal or mediastinal disease was based on the presumed diagnosis. One child had a retained tracheal foreign body. One child had an extruded esophageal foreign body with secondary development of an obstructing mediastinal granuloma. The child with mediastinal lymphoma underwent chemotherapy with marked, rapid improvement in airway symptoms. The 2 children with double aortic arches underwent successful surgical correction with improvement in both the postoperative history and endoscopic findings. Two children with innominate artery compression received a pediatric thoracic surgical consultation, but neither family agreed to aortopexy. The long-term results of the recommendations for all of the children in this series are currently being evaluated and are not included in this study.

CLINICAL PERSPECTIVE

Our data indicate that the clinical significance of recurrent intermittent airway obstruction in children, as well as the utility of aerodigestive tract endoscopy, is potentially underappreciated by both primary care physicians and pediatric subspecialists. Thirty-nine children had the potential significance of their "recurrent croup" history first identified during a routine general pediatric otolaryngology consultation. These children were not specifically referred for an "airway evaluation" or for a history of "croup," "stridor," or "chronic cough." Rather, their consultation was requested for a history of otitis media with effusion, "chronic sinusitis," or an obstructive *upper* conductive airway concern. We believe that many children with a history of "recurrent croup" do not obtain an accurate diagnosis and hence receive inadequate therapeutic intervention. Otolaryngologists, by virtue of their endoscopy training and sensitivity to the potential development of acquired laryngotracheal stenosis, as well as the general nature of pediatric otolaryngology referrals, are in a unique position to proactively identify those children with a significant history of recurrent upper airway obstruction and initiate or advance their diagnostic evaluation. With a proper diagnosis, clinically appropriate management can be determined and carried out in a timely manner.

The term "spasmodic croup" has been used in the otolaryngology literature for more than 30 years without clear definition. It has been used to describe children with "allergic mucosa" who develop marked airway mucosal reactivity with minimal precipitant insult.[8] The term has also been used to describe recurrent laryngeal spasm. In general, these children are afebrile and experience sudden onset of inspiratory stridor and respiratory distress, usually at night.[9] As described, recurrent episodes are not uncommon, and spontaneous recovery should be anticipated. The cause of their laryngeal spasm or "tracheal cough" most likely results from gastroesophageal reflux or atopic mucosal inflammation[10] — collectively the two most common findings in our review. We believe that these cases represent a subset of those cases labeled as "recurrent croup," which itself falls within the broader diagnostic umbrella of "chronic cough."[11]

In our experience, primary care physicians are receptive to recommendations for aerodigestive tract endoscopic evaluations in children with recurrent airway obstruction histories. On occasion, the child may have undergone a barium esophagram or chest radiography, but in retrospective discussion with the referring physician, we found that their differential diagnosis was usually limited to laryngomalacia, congenital cardiovascular anomalies, or "asthma." Using image documentation of endoscopy findings in the patient's chart, in physician correspondence, and in presentations at educational forums has helped improve interspecialty cooperation in the diagnosis and management of children with "recurrent croup." We have noticed an increase in the number of referrals initiated by primary care physicians, intensivists, pulmonologists, and anesthesiologists as they have become more aware of the potential clinical significance of the history and findings in these children. An ongoing outcome analysis of our caseload is being performed to evaluate the clinical impact of obtaining an accurate diagnosis and the resultant clinical value of directed therapeutic intervention in these children.

SUMMARY

Recurrent intermittent airway obstruction in children is an underappreciated clinical scenario. "Recurrent croup" is not a diagnosis for these children. A history of recurrent or episodic airway obstructive signs and symptoms should alert the otolaryngologist to the potential for intrinsic or extrinsic laryngotracheal disease that can compromise the pediatric airway. A thorough history and physical examination, including airway and esophageal endoscopy, is an integral part of the diagnostic evaluation. The decision and timing for an endoscopic evaluation is best determined after analysis of the patient's age, past medical history, clinical severity, and suspected diagnosis. Concerted effort with other specialists is often required. By virtue of his or her knowledge of aerodigestive tract anatomy and endoscopy skills, the otolaryngologist can take a proactive role in arriving at an accurate diagnosis in these children.

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Ann Otol Rhinol Laryngol 110:2001

ASSOCIATION OF LARYNGOPHARYNGEAL REFLUX DISEASE AND SUBGLOTTIC STENOSIS

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Laryngopharyngeal reflux (LPR) disease and extraesophageal manifestations of gastroesophageal reflux have been recognized to have dramatic effects in the upper airways. Patient-reported symptoms alone underestimate the presence of LPR, making accurate clinical diagnosis difficult. Many previous studies examine populations with only standard dual-probe pH testing that does not include a test probe in the pharynx. Therefore, documentation of acid exposure at the laryngeal inlet is lacking. In adult patients with subglottic stenosis (SGS), whether due to granulomatous disease or presumed idiopathic causes, LPR is often a contributing or causative factor. A retrospective chart review from 1991 to 1999 identified 19 patients with SGS. Ten of the 19 patients had concomitant disease states, including sarcoidosis (3), Wegener's granulomatosis (3), laryngeal trauma (3), and a history of intubation (1). Fourteen patients underwent 24-hour ambulatory pH probe testing with 3- or 4-port probes. The proximal port in either catheter was positioned by manometric guidance directly behind the laryngeal inlet. Measurements of pH of less than 4 were recorded at the level of the larynx in 12 of the 14 patients tested (86%). This finding was noted in half of the patients despite empirical therapy with proton pump inhibitors at the time of the testing. Seven of 10 patients with underlying disease were studied, and all demonstrated acid reflux in the hypopharynx. In 9 patients, the stenosis was presumed to be idiopathic. Five of the 7 patients (71%) with idiopathic SGS tested had positive pH probe studies (pH below 4 in the pharyngeal probe). Our results demonstrate a strong association of LPR and SGS. In the idiopathic group, reflux is the probable cause of their stenosis. In the group of patients with underlying disease states, reflux was involved in all tested patients and likely acts as a synergistic factor that stimulates their granulomatous disease to react and subsequently result in the development of stenosis. Evaluation for LPR with pharyngeal pH testing should be performed in all patients with SGS.

KEY WORDS — extraesophageal reflux, gastroesophageal reflux, granulomatous disease, idiopathic subglottic stenosis, laryngopharyngeal reflux, pH monitoring, sarcoidosis, subglottic stenosis, Wegener's granulomatosis.

INTRODUCTION

The causes of subglottic stenosis (SGS) compose a heterogeneous group of disorders both acute and chronic in nature. Endotracheal intubation or laryngeal trauma can acutely restrict the size of the subglottic airway. The incidence of SGS related to intubation ranges from 1% to 11%. Traumatic intubation and reintubation, as well as tube size, failure to firmly secure the tube, and systemic factors of sepsis and hypotension, have all been implicated as contributing factors.[1-3] The larger category of acquired SGS is not as well understood. This encompasses subgroups of autoimmune and granulomatous disorders, such as Wegener's granulomatosis (WG) or sarcoidosis, and an idiopathic group.[4] Host susceptibility factors are poorly understood in all 3 categories.

Subglottic stenosis in which the cause of scarring and narrowing remains unknown has been termed idiopathic SGS.[3-7] The patient population is predominately female. Previous reviews have postulated that this group may represent a distinct clinical entity, a complication of poorly understood systemic illness, or infection.[4] A distinct identifiable cause was not suggested until Bogdasarian and Olson,[8] in 1980, described a case of posterior SGS. In their report, for the first time, gastroesophageal reflux disease (GERD) was listed as a possible contributing factor to upper airway disease. Bain et al,[9] in 1983, similarly described a female patient without predisposing factors who developed a significant SGS. Tomography revealed an airway the "size of a pencil eraser." She subsequently underwent open Nissen fundoplication and endoscopic laryngeal repair, dilation, and Kenalog injection with tracheostomy. Five months after the surgery, she had a normal upper airway.[9]

This experience set the stage for further investigation into reflux effects on the subglottis. Borowiecki and Croft[10] had already shown that subglottic injury in dogs could lead to SGS. Dogs given only mucosal lesions had rapid reepithelialization. Their findings revealed that subglottic injury did not progress to a stenosing lesion unless the perichondrium was damaged. They postulated that initiation of stenosis formation began with a reparative reaction that

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Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

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followed an injury. With necrosis of the cricoid cartilage, fibrous repair caused scar formation and eventual stenosis. The animals were observed for as long as 3 months and tolerated the slow expansion of the resultant stenosis. Only when the subglottic diameter decreased to 3 to 4 mm did they become symptomatic.[10] Little et al[11] then expanded on this model with the application of gastric acid to a traumatized subglottis. Gastric acid was applied for 1 minute every other day. In this study, they were able to demonstrate that the addition of gastric acid in the setting of (only) mucosal injury led to perichondritis, chondritis, and subsequent stenosis.[11] In spite of these studies, application of this information clinically has been inconsistent. The majority of the work in human adults has been done by Koufman,[3] who in 1991 studied 182 consecutive patients, 32 of whom had SGS. Of these patients, 78% demonstrated abnormal reflux on pH monitoring.[3] Despite this information, the term idiopathic SGS continues to be widely used.

Other investigations centered initially on the effect of reflux on the pediatric airway.[2,6,7,12] It is known that neonates have an extremely high incidence of gastric reflux (15% to 86%). At birth, infants do not have a demonstrable lower esophageal sphincter (LES). Within 2 months, an LES can be discerned. The intervening predisposition to reflux is thought to cause multiple upper airway complications, including SGS, recurrent croup, sleep apnea, chronic cough, and possibly sudden infant death syndrome.[7]

Perhaps the link between the effects of gastric acid on the subglottis in adults was not readily identified because of the subtle clinical findings. Sixty percent of laryngeal patients do not have classic symptoms of heartburn.[3] Patients often deny hoarseness or sore throat. Instead, chronic cough (usually nocturnal), progressive dyspnea on exertion (DOE), adult-onset asthma, and eventual audible stridor represent a typical constellation of presenting symptoms. Long-term acid reflux into the tracheobronchial tree has also been linked to idiopathic pulmonary fibrosis.[13] In that study, 94% of patients with idiopathic pulmonary fibrosis had abnormal acid exposure in the distal and/or proximal esophagus. Also, 75% of patients did have abnormal reflux without the typical GERD symptoms of heartburn or regurgitation.[13] The term laryngopharyngeal reflux (LPR) has been used by Koufman et al[14] and others[15] to describe these distinct differences between otolaryngological and gastroenterologic patients manifesting acid reflux.

Strides toward accurate diagnosis have encouraged the use of 24-hour pH probe monitoring to document reflux episodes.[3,5,13,16-18] However, the use of pH monitoring in this subgroup of patients remains sporadic. Dual-probe pH testing remains the most widely used, with electrodes located just above the LES and below the upper esophageal sphincter (UES). Probe placement between studies may be variable or inconsistent.[6,7,19] With 4-port pH probes, an electrode located above the UES gives unique information about acidification at the larynx.[13,16-18] Many studies still do not consistently employ such a pharyngeal port.

In patients with granulomatous disorders such as WG, SGS may take on particular severity. Wegener's granulomatosis is a disorder of necrotizing granulomatous inflammation affecting the upper airway, lungs, and kidneys. Certain patients may present with isolated laryngeal involvement, typically affecting the subglottis. An overall incidence of SGS in WG has been reported at only 8.5% to 10%.[20,21] Despite this relatively low incidence, tracheostomy is required in as many as 52% of patients in some series, with decannulation rates varying between 0% and 86% after multiple procedures.[21] Women with WG are twice as likely to have SGS as men with WG.[20] It has not been clear why only certain patients with WG are affected by SGS.

The goal of this study is to describe a population of patients presenting with SGS. The appropriate clinical evaluation, the impact of comorbid disease states, and treatment are discussed.

MATERIALS AND METHODS

A retrospective chart review was undertaken for the years 1991 to 1998 of patients with SGS who presented to the University of Washington Medical Center Voice Disorders Clinic. Each patient underwent documentation of his or her history and a thorough head and neck examination. Symptoms of LPR were assessed by questioning for hoarseness, chronic cough, nocturnal cough, globus pharyngeus, throat clearing, postnasal drip, excessive mucus or phlegm, sore throat, heartburn, indigestion, chest pressure, regurgitation, abdominal bloating, belching, dysphagia, foul taste in the throat, halitosis, and antacid use. Additionally, signs of DOE and audible stridor were recorded. Patients who carried a diagnosis of adult-onset asthma were identified. A rheumatology consultation was obtained in those patients known or believed to have contributory disease states. Flexible laryngoscopy with videostroboscopy was performed to evaluate the extent of the subglottic lesion.

The patients were subsequently evaluated with 24-hour pH probe monitoring with 4 ports regardless of the presence or absence of LPR symptoms. The pH probe (Medtronics Synectics, Shoreline, Minnesota)

TABLE 1. PATIENT DEMOGRAPHICS

	No. of Patients	*Male:Female*
Idiopathic	9	1:8
Wegener's granulomatosis	3	1:2
Sarcoidosis	3	0:3
Trauma	3	0:3
Intubation	1	0:1
Total	19	2:17

consisted of 4 monocrystalline antimony electrodes spaced 5 cm apart and embedded within a single catheter. The pH electrodes were calibrated at 37°C in pH 7.0 and 1.0 buffer solutions (Medtronics Synectics) before and after each study. Manometric guidance to identify the LES and UES was used in each patient. Peristaltic activity of the esophagus was recorded during manometry.

The 4-channel pH probe was placed with the most proximal channel 2 cm above the UES, that is, at the level of the laryngeal inlet. The remaining ports, each spaced 5 cm apart, were placed at distal, middle, and upper portions of the esophagus. The most distal port was located 13 cm below the manometrically identified UES. Identification of the UES by manometry allowed for placement of 1 electrode 1 cm below this valve. Monitoring consisted of simultaneous recording in all channels. The pH electrodes were connected to a portable digital data recorder (Micro Digitrapper Mark III, Medtronics Synectics) that stores data every 4 seconds for 24 hours. The patients were instructed to appropriately log meals, coughs, and supine versus upright periods via a written diary. The use of acid suppressive medications was also noted. Catheter removal was accomplished the following day after 20 to 24 hours of pH recording. Portable digital data recordings were downloaded into a computer and analyzed by Esophagram software (Medtronics Synectics). Pharyngeal reflux was defined as a drop in the pharyngeal channel to a pH of less than 4 that occurred simultaneously with acidification of the distal esophagus.

Operative therapy, when needed, consistently included diagnostic laryngoscopy and bronchoscopy. Serial dilation with rigid bronchoscopes was the preferred method for alleviation of stricture. Subsequent injection with Kenalog or topical application of mitomycin occurred in appropriate subjects. Some patients did not need surgical intervention because the size of their airway was adequate. Others underwent multiple or complex reconstructive procedures to ensure airway patency.

RESULTS

Nineteen patients were identified with SGS. The group consisted of 2 men (ages 30 and 83 years) and 17 women (ages 28 to 76 years). The causes of stenosis were idiopathic (9 patients), WG (3 patients), sarcoidosis (3 patients), trauma (3 patients), and intubation (1 patient; Table 1). Fourteen patients underwent pH testing. Four patients were not tested early in the study because of a lack of routine testing capabilities. One additional patient had concomitant medical issues that prohibited testing. All patients were able to tolerate probe placement for the entire length of the study period.

TABLE 2. COMPARISON OF PATIENTS WITH IDIOPATHIC SUBGLOTTIC STENOSIS VERSUS STENOSIS FROM OTHER CAUSES

Variable	*Idiopathic*	*Other Causes*
Patients (No.)	9	10
Mean age (y)	56.8 (35-83)	46.3 (28-70)
Female patients (No.)	8	9
No. of procedures	16	19
pH < 4 on proton pump inhibitors	2	3

In order to effectively compare patient demographics and symptoms, the patients were divided into 2 groups: those with presumed idiopathic causes for their SGS (9 patients) and those with other disease states as causes for the SGS (10 patients). The mean age was 56.8 years in the idiopathic group, versus 46.3 years in patients with other disease states. The number of operative procedures undertaken to provide airway patency accounted for 16 in the idiopathic category and 19 in the setting of other disease (Table 2).

The 2 patient groups were considerably different in terms of symptoms. Both groups uniformly complained of DOE (100% of patients). Seven of the 10 patients (70%) with identified causes for their SGS experienced stridor and cough. Heartburn was a feature in 60% (Table 3). Conversely, none of the patients in the idiopathic SGS category complained of heartburn. In this patient population, the preponderant symptoms included cough (77%), excessive mucus (44%), and stridor (44%; Table 4). Adult-onset asthma was a diagnosis also seen exclusively in the idiopathic group (44%).

Measurements of pH of less than 4 were recorded at the level of the larynx in 12 of the 14 patients tested (86%). Of the 10 patients with underlying disease states, 7 patients were tested. All 7 (100%) had acid exposure in the laryngeal port. Additionally, 4 of the 7 patients were taking proton pump inhibitors at the time of their pH probe study. In these 4 patients (57%), therapy with proton pump inhibitors did not suppress acid events in the pharynx.

In 9 patients, there was no history of rheumatologic disorders, intubation, or trauma, and thus, the SGS

TABLE 3. SYMPTOMS OF PATIENTS WITH SUBGLOTTIC STENOSIS FROM "OTHER" (NON-IDIOPATHIC) CAUSES

	Patient										
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*Total*
Heartburn	+		+	+		+	+		+		6
Dyspnea on exertion	+	+	+	+	+	+	+	+	+	+	10
Hoarseness			+	+			+			+	4
Throat clearing		+			+		+				3
Globus pharyngeus			+		+		+				3
Cough	+	+	+		+		+	+		+	7
Mucus	+										1
Stridor	+	+	+	+		+			+	+	7
Dysphagia											0
Regurgitation			+				+				2
Sore throat							+				1

was presumed idiopathic. Seven of these patients underwent pH testing; 5 of these 7 (71%) exhibited acid reflux to the level of the pharynx. Again, 3 of the 7 patients (43%) had breakthrough acid exposure measured at the laryngeal inlet while on proton pump inhibitor therapy.

All patients had an SGS that narrowed the normal airway diameter by 30% to 90%. Stenosis was graded during office-based laryngoscopy and during operative intervention. The Figure documents the preoperative airway stenosis in a patient with a presumed idiopathic cause and the subsequent postoperative result following serial dilation.

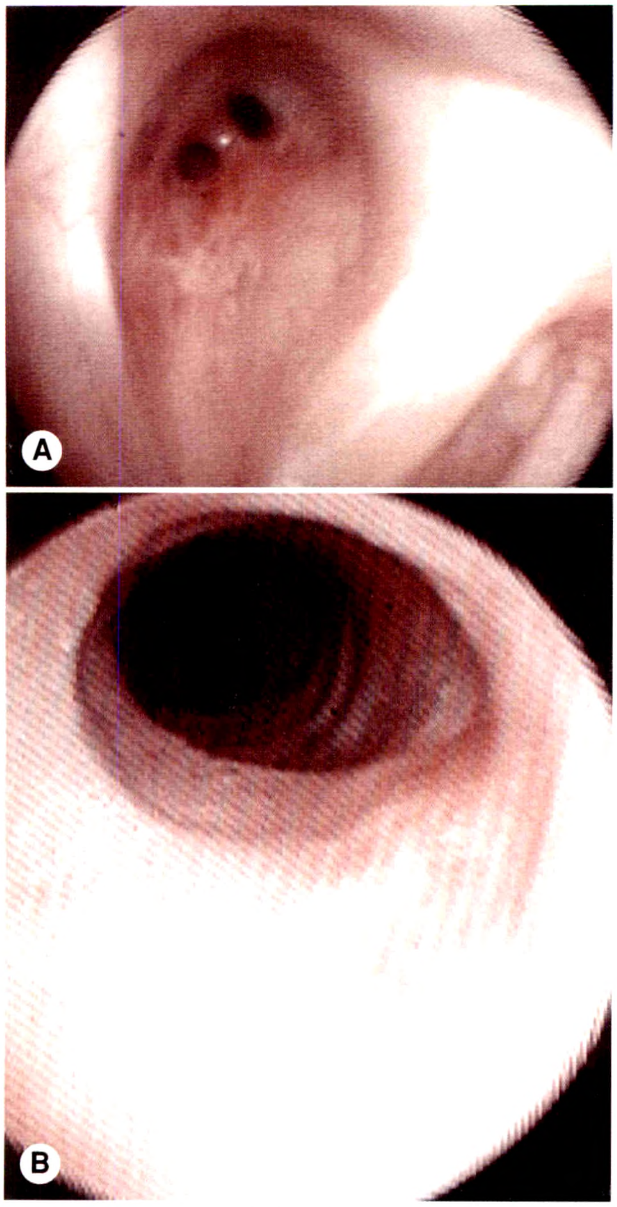
DISCUSSION

Subglottic stenosis involves a progressive narrowing of the airway in the setting of a chronic disease state or of a presumed idiopathic cause. Laryngopharyngeal reflux has been implicated but is not fully accepted as the instigating agent in those cases of SGS labeled as idiopathic.[3,6] With the routine use of ambulatory 4-channel pH probe monitoring, acid reflux can reliably be shown to be present at the laryngeal inlet. In this study, we found that patients with SGS had a 70% to 100% incidence of measurable acid at the hypopharynx. Other manifestations of extraesophageal reflux, such as pulmonary changes, have been previously noted by other authors.[3,13,22-24] These studies identify reflux as a cause in 80% of cases of adult-onset asthma, 75% of cases of chronic hoarseness, and at least 20% of cases of chronic cough.

Otolaryngological manifestations of granulomatous disease occur at initial presentation in 73% of patients, and during the course of the disease in 92% of patients.[20,21] De Vries et al[25] have postulated that circulating autoantibodies against neutrophil constituents may cause direct subglottic inflammation and resultant stenosis. These authors did not consider the role of LPR in initiation and progression of the subglottic inflammation. Patients with granulomatous disease are known to have a small-vessel vasculitis that is presumably immunologically mediated.[20,21] Also, patients younger than 20 years of age have an increased incidence of SGS. The reason for this preponderance is unclear.[21] With vasculitis in the setting of an active inflammatory state, resultant decreased healing ability can be expected. However, the cause of the inflammatory state has not been adequately explored. In these immunologically affected patients, the reflux potentiates their underlying inflammatory state, which initiates cartilaginous inflammation, followed by resultant scarring second-

TABLE 4. SYMPTOMS OF PATIENTS WITH PRESUMED IDIOPATHIC SUBGLOTTIC STENOSIS

	Patient									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*Total*
Heartburn										0
Dyspnea on exertion	+	+	+	+	+	+	+	+	+	9
Hoarseness	+				+					2
Throat clearing		+	+		+					3
Globus pharyngeus			+		+					2
Cough	+	+	+	+	+	+	+			7
Mucus	+	+	+						+	4
Stridor	+			+	+				+	4
Dysphagia					+					1
Regurgitation										0
Sore throat							+			1



Patient with presumed idiopathic subglottic stenosis. **A)** Before operation. **B)** Two months after endoscopic dilation and proton pump inhibitor therapy.

ary to the underlying vasculitis. This process is similar to that which occurs in the nasal septum. In our study, the different causes of SGS, idiopathic versus other comorbid states, revealed a 10-year age difference at initial presentation. This finding supports the mechanism that patients with WG form stenoses more aggressively and at an earlier age because of the inflammatory vasculitis potentiating scar formation. The patients with presumed reflux-induced stenosis likely develop subglottic scarring at a slower rate, without the contribution from vasculitis, and thus present at an older age.

Twenty-four-hour pH monitoring has revolutionized our ability to consistently and reliably identify acid exposure in the hypopharynx and esophagus. Prior testing modalities, including barium esophagography and esophagogastroduodenoscopy (EGD), are suboptimal at diagnosing reflux.[3] Barium esophagography can detect a hiatal hernia, which predisposes to reflux and true esophageal or antral erosions. However, reflux to the level of the pharynx is considered an intermittent event that primarily occurs in the upright as opposed to the supine position. Because of the intermittent nature of its occurrence, a single study encompassing only several minutes may fully miss important reflux episodes. Thus, the overall specificity of barium esophagography for reflux diagnosis is only 30%.[3,26] The same holds true for EGD. Frank erosive changes are seen only in the minority of patients with LPR.[3] Thus, normal examination findings may be reported by the gastroenterologist despite LPR symptoms and SGS evident in the patient. An EGD with only normal to mild findings speaks to the intermittent nature of extraesophageal acidification versus the long-term esophageal pooling that can be seen in classic GERD. Use of 24-hour pH probe monitoring with a pharyngeal port before and after the institution of treatment should be considered the best diagnostic regimen.[3,18] Even with the sophistication of 4-channel pH monitoring, diagnosing reflux can be difficult. The 24-hour monitoring requires patient compliance and adherence to standard lifestyle and eating habits. A patient's failure to eat his or her normal meals or partake of his or her regular physical activity because of discomfort from the catheter would significantly blunt normal acid secretion. A negative study should still be questioned and carefully interpreted by the ordering physician in a case of SGS from any cause.

For better interpretation of pH monitoring, patient symptoms must be aggressively sought. In this study, the population of idiopathic SGS patients revealed considerably more adult-onset asthma diagnoses than did those patients with comorbid conditions. Also, none of the patients in this category admitted to any heartburn symptoms despite specific questioning. The major complaints included DOE, cough, and increased mucus secretion. The symptom of stridor was more evident in the group with other disease states. Much as Borowiecki and Croft[10] demonstrated in their canine studies, the gradual development of SGS is very well tolerated. These lesions are typically stable and show no evidence of acute inflammatory changes. Thus, patients often tolerate even quite minimal airways without significant distress.

In the setting of a life-threatening complication, the tendency is toward aggressive management. Early tracheostomy, laser resection of the subglottic tissue, and laryngotracheal reconstruction have been discussed as primary surgical modalities.[21,27] Our study concurs with that of Koufman,[3] in that we have been able to show that therapy with serial dilation and avoidance of tracheostomy provides efficacious airway management. The patients who required multiple procedures were often initially treated with laser resection. Laser damage at the subglottis likely furthers the already active inflammatory state and worsens the stenosis. Similarly, open repair exposes the already traumatized cartilage and may potentiate subsequent scar tissue reaction. Tracheostomy was used in only 2 patients, who had active progression of their granulomatous disease despite maximal chemotherapy. Use of a tracheostomy should be avoided whenever possible.[3] The tracheostoma has underlying chronic infection despite the best attempts at local wound care and outwardly clean appearance. This chronic inflammation likely potentiates the subglottic process, preventing final healing and relief of inflammation. With concomitant medical therapy and, if necessary, surgical management of reflux-induced SGS, endolaryngeal dilation allows for consistent, long-lasting results in the vast majority of patients.

Breakthrough reflux occurs during maximal medical management and requires a high degree of clinical suspicion for appropriate identification and management. Koufman[3] found a significant failure rate (55%) of medical antireflux therapy with H2 blockers. Forty-one percent of his stenosis group eventually needed Nissen fundoplication for improved acid control.[3] Omelanczuk et al[17] also obtained pH probe measurements in patients with laryngeal symptoms before and after proton pump inhibitor therapy. One patient of 8 continued to have pharyngeal episodes on double-dose proton pump inhibitor therapy. Furthermore, 3 of the 8 patients had continued to have abnormal distal DeMeester scores while on double-dose therapy. We agree with Koufman that acidification at the laryngeal inlet continues to occur frequently despite maximal medical therapy. The failure of even aggressive medical therapy is due to the severe effect acid has outside the esophagus, and 1 or 2 acid events in the larynx and trachea are probably enough to sustain the chronic effects of acid-induced inflammation and injury. The group of patients with SGS appears to be at particularly high risk. In this study, empirical use of antireflux medication was with proton pump inhibitor therapy rather than the H2 blockers that were used by Koufman. Even in this drug class, believed to provide far more effective acid blockade, frequent breakthrough reflux was identified.

This study demonstrates that in cases previously described as idiopathic, laryngotracheal reflux was likely the major cause of the SGS in 70%. Given this finding, the label of idiopathic SGS should be replaced by reflux-induced SGS. The possibility of reflux contributing to any stenosis, regardless of the presumed cause, should be vigorously investigated with 24-hour pharyngeal pH probe testing before and after the institution of medical therapy. This approach is particularly important in those patients with other disease states in which laryngotracheal reflux can provide the inflammatory injury that is the underlying event that triggers the progression and persistence of the SGS.

CONCLUSIONS

1. The label of idiopathic SGS should be replaced by reflux-induced SGS. Without other clear causes, reflux should be considered the cause of the stenosis and maximally treated until proven otherwise.
2. Twenty-four-hour pH probe monitoring with a pharyngeal port before and after the institution of treatment should be considered the diagnostic regimen of choice.
3. Breakthrough reflux while on maximal medical management occurs and requires a high degree of clinical suspicion for appropriate identification and management.

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TRACHEOESOPHAGEAL PUNCTURE IN THE OFFICE SETTING WITH LOCAL ANESTHESIA

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Tracheoesophageal puncture (TEP) with voice prosthesis placement is currently the method of choice for vocal rehabilitation of patients who have undergone total laryngectomy. Occasionally, secondary TEP needs to be performed. We have used a TEP technique that is performed in the clinic setting with local anesthesia and no sedation. The purpose of this study was to review our technique and experience and to evaluate results, complications, and patients' acceptance of the procedure. We performed a retrospective chart review of the records of 14 patients who had undergone total laryngectomy and secondary TEP placement in the clinic setting. The procedure was well tolerated. The voice results were fair to good in 11 of 12 patients. There was 1 complication, a false passage between the trachea and the esophagus. Voicing was immediate in 12 of the 14 cases. We conclude that TEP can be performed in the office setting with local anesthesia. The voice results are excellent, and the procedure is well tolerated by the patient. Proper patient selection and regular follow-up by a speech-language pathologist are important.

KEY WORDS — laryngectomy, secondary tracheoesophageal puncture, tracheoesophageal puncture, vocal rehabilitation.

INTRODUCTION

Tracheoesophageal puncture (TEP) with voice prosthesis placement is currently the method of choice for vocal rehabilitation of patients who have undergone total laryngectomy. It is most commonly done as a primary procedure. Secondary TEP is performed when a primary TEP has failed or when for technical or medical reasons TEP cannot be performed at the time of the initial laryngectomy. Many modifications of secondary TEP procedures have been described.[1-9] These include percutaneous gastrostomy sets, various introducer kits, and large clamps. Secondary procedures usually require intravenous sedation or general anesthesia. Rigid or flexible endoscopy is often used. The majority of techniques describe placement of a catheter at the time of the procedure with subsequent change to a voice prosthesis 2 or 3 days later. Because of these factors, secondary TEP is usually performed in the operating room, and patients require a subsequent office visit before being able to vocalize.

We have used a TEP technique that is performed in the clinic setting. Local anesthesia with no sedation is used. The advantages of this technique include ease of scheduling and, since the voice prosthesis is placed at the time of the procedure, immediate vocalization by the patient. The objectives of this study were to describe our technique and to evaluate the voice results, patient tolerance, and complications.

MATERIALS AND METHODS

A retrospective chart review was performed of patients who had undergone total laryngectomy at the Portland Veterans Administration Medical Center and Oregon Health Sciences University Hospital from 1994 to 2000 and who used a voice prosthesis. Fourteen patients who had undergone a secondary TEP placement in the clinic setting were identified and are the subject of this report (13 men and 1 woman). The average age was 65 years (range, 44 to 77 years). The follow-up ranged from 2 months to 4 years, with a mean of 14 months and a median of 5 months. Eight patients (57%) underwent radiotherapy either before or after the operation. Secondary TEP was performed a median of 6 months after total laryngectomy (Fig 1). The major reason for performing a secondary TEP was the inability to replace a prosthesis or prosthesis loss (6 patients; see Table). Six patients did not undergo a primary TEP at the time of the total laryngectomy for the following reasons: chondritis at the time of total laryngectomy (1 patient), reconstruction of the large pharyngeal mucosal defect with a pectoralis major myocutaneous flap (1 patient), a combined total glossectomy with total laryngectomy (1 patient), and healing concerns (3 patients). Persistent fistula, leakage, and feeding difficulties led to

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Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

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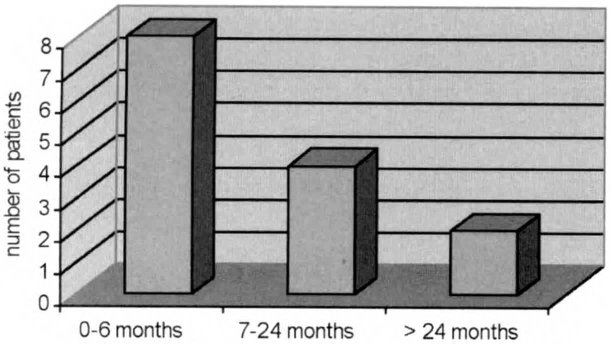

Fig 1. Interval after total laryngectomy.

REASONS FOR SECONDARY TEP PLACEMENT

	No. of Patients
Previous TEP history	
Prosthesis removal or tract closure	6
Voice prosthesis removed because of fistula	1
TEP closed during radiotherapy	1
Total	8
No history of primary TEP	
Tumor size and reconstruction concerns	2
Healing concerns	4
Total	6
Total patients	14

TEP — tracheoesophageal puncture.

removal of an existing voice prosthesis with subsequent TEP closure in 1 patient. One patient's tract closed during radiotherapy.

The Cook TEP kit was used in this study (Fig 2). With the patient seated in the examination chair, 4% lidocaine spray is used to anesthetize the oropharynx and oral cavity. Then, 1% lidocaine with epinephrine is injected at the site of the planned TEP. The site of the planned TEP is decided upon by the otolaryngologist and the speech therapist. An esophageal dilator (28F or 32F) is lubricated and swallowed by the patient. A 16-gauge needle is inserted at the site of the TEP with the bevel oriented inferiorly. The needle is inserted into the esophageal bougie located in the esophagus. Gentle movement of the bougie confirms the location of the needle (Fig 3). The needle is gently dislodged from the bougie, and the bougie then is withdrawn. A guide-wire is passed into the esophagus through the needle. Location of the wire in the esophagus is confirmed by flexible nasopharyngoscopy. If there is any resistance to passing the guide-wire, the needle is withdrawn and the procedure is started over. A dilator is passed over the guide-wire and left in place for several minutes. The wire and the dilator are then withdrawn as a unit, and a Blom-Singer voice prosthesis dilator is placed. This dilator is left in place for 5 minutes and then removed. After measurement for the proper size, a Blom-Singer voice prosthesis is placed. Patients are instructed in the use and function of the prosthesis for speech therapy. Commonly, patients are able to speak immediately.

RESULTS

Twelve of the 14 patients retained their prosthesis at the time of last follow-up. In 11 of the 12 patients who had voice evaluations, the voice was rated as fair to good, with 85% to 100% intelligibility as rated by our speech pathologist. A patient who underwent a combined total laryngectomy and total glossectomy had poor intelligibility. One patient had good voice results but did not use the prosthesis on a regular basis and preferred the electrolarynx. One patient was unable to care for the prosthesis, and his vocalization was poor because of abundant secretions.

There was a single complication in our series. In 1 patient, a false anterior passage was created during placement of the voice prosthesis dilator and was recognized at the time the dilator placement was attempted. Although we could pass the guide-wire into

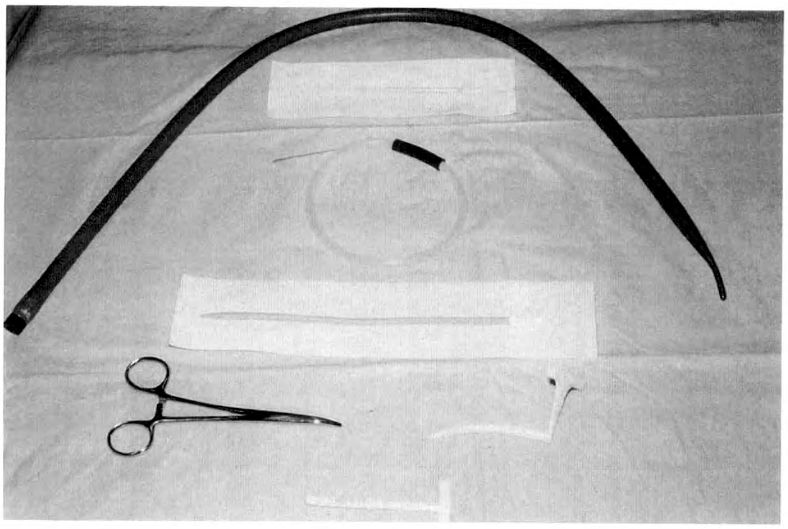

Fig 2. Equipment used to perform tracheoesophageal puncture.

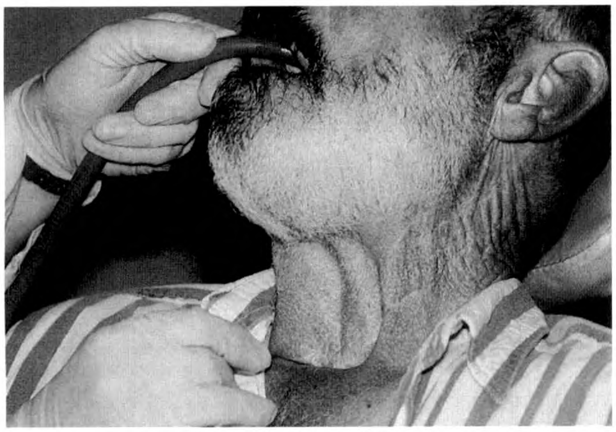

Fig 3. Needle is inserted, and needle position is confirmed by gentle traction on bougie.

the esophagus, we could not place the dilator. The complication was managed by placement of a red rubber catheter to mature the fistula for 2 weeks. Prophylactic antibiotics were administered for 2 weeks. A Blom-Singer voice prosthesis was placed 2 weeks later with no further difficulties.

DISCUSSION

Laryngeal cancer is the second most common head and neck malignancy. It is estimated that there will be 11,600 new cases diagnosed each year and that 4,250 patients will die each year from this disease. In 1873, Billroth successfully performed the first total laryngectomy for cancer.[10] After that procedure, the need for vocal rehabilitation was recognized. Since that time, multiple devices and reconstructive techniques to facilitate vocal rehabilitation have been described. These have included written communication, mechanical devices such as electrolarynxes, esophageal speech, and, recently, TEP with a voice prosthesis. Singer and Blom first described TEP in 1980.[11,12] They introduced a "duckbill" prosthesis. Initially, experience with the TEP as a secondary procedure was in patients in whom esophageal speech failed or in patients who were dissatisfied with their electrolarynx. Over time, surgeons gained experience with the procedure, and its safety was established.[13] It is now most commonly performed as a primary procedure. Secondary TEP is used when primary TEP fails or when primary TEP is precluded for technical or medical reasons. Primary TEP failure is usually due to prosthesis removal with an inability to replace the prosthesis. Occasionally, healing concerns or feeding problems lead to the removal of the voice prosthesis with subsequent TEP closure. Concerns about postoperative healing or the need for radiotherapy may prevent some surgeons from performing a primary TEP. Lack of patient motivation may also be a factor in failure to perform a TEP.

Tracheoesophageal puncture is not a completely benign procedure; the complications include tracheostomal stenosis, tracheal granulations, prolapse of esophageal mucosa, leakage at the TEP site, aspiration of the prosthesis, and inadvertent TEP closure.[14,15] Complications of placement include creation of a false passage, esophageal injury, cervical cellulitis, cervical spine fracture, and mediastinitis.[16] Other complications described include aspiration pneumonia, death from aspiration, esophageal stenosis, and fistula migration.[14]

Multiple methods for secondary TEP have been described in the literature.[1-9] The majority of them are performed in the operating room with general anesthesia. A few authors have reported their experience in an office setting; however, intravenous sedation was used. Our method is performed with topical lidocaine spray and local lidocaine injection, without the use of intravenous sedation, and is well tolerated by patients. With proper local anesthesia, patients tolerate the esophageal bougie and percutaneous puncture. In fact, in a few cases in which patients have removed the prosthesis and unsuccessful attempts were made to reinsert the prosthesis, the patients found the percutaneous insertion easier, less painful, and easier to tolerate than the forceful reinsertion. Proper placement of the prosthesis is confirmed at the time of the procedure by flexible nasopharyngoscopy. Occasionally, suction is required to clear secretions from the pharyngeal segment. The key is to introduce the guide-wire with no resistance; if there is any resistance, we start over. Most patients are able to start communicating by using the prosthesis right after its placement. Patients are counseled immediately in the use of their prosthesis, since the procedure is performed with a speech therapist present.

Our small series demonstrated a single complication. This was the creation of a false passage between the trachea and the esophagus. It did not occur during the placement of the dilator with the percutaneous kit, but in fact, occurred when the inserter was placed. This patient had an uneventful insertion of a red rubber catheter with delayed placement of the voice prosthesis in 2 weeks. He has since done well, and the site has healed with no complications. Since the procedure is performed with visualization of the shunt, guide-wire, and dilator, and since a bougie is used to protect the posterior pharynx, many of the complications that can occur with introduction of an esophagoscope and general anesthesia are prevented. This technique has proven to be relatively easy to teach and to execute.

Finally, performing these punctures, in all cases, at a scheduled office visit facilitated acceptance by

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the patients and eased the scheduling, as the procedures did not have to fit into an operating room schedule.

CONCLUSIONS

Tracheoesophageal puncture under local anesthesia is well tolerated in the clinic setting. There was 1 complication in our series. Proper patient selection is important, as evidenced by the 2 instances of failed procedures in our review. The voice results are fair to good and are comparable with those obtained with a voice prosthesis placed by the standard method or by primary TEP. Voicing is usually immediate, although further adjustments are sometimes necessary, and regular follow-up by a speech-language pathologist is important.

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PEDIATRIC LARYNGOSCOPES: DESIGN AND APPLICATION

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Examination of the upper aerodigestive tract for diagnosis and treatment has become more important in pediatric otolaryngology. Techniques of endoscopy have improved, anesthesia is safer, and there are many specialized laryngoscopes designed specifically for the small, sensitive airways of infants and children. Knowledge of the design advantages, specifications, and applications, including the use of telescopes and the operating microscope, will assist in selection of the appropriate laryngoscope for optimum exposure.

KEY WORDS — laryngoscope, laryngoscopy.

Development of direct laryngoscopy probably began with Green[1] (1852), who used sunlight and a bent spatula. Killian[2] (1912) originated the suspension laryngoscope, which was later modified and popularized in the United States by Lynch.[3] Early in the 20th century, Jackson[4] applied distal lighting to his endoscopic equipment, and with further contributions by Paul Holinger in Chicago and Sir Victor Negus[5] in London, examination of the adult larynx by direct laryngoscopy became well established.

On the other hand, there is little information about the development and application of laryngoscopy for pediatric patients; it seems that at first, small adult or anesthetic laryngoscopes were used. In recent times, as new, diverse, and distinct congenital and acquired diseases have been recognized, the need for diagnostic endoscopy has grown along with improved methods of anesthesia. It became obvious that there was a requirement for specialized endoscopic instruments specifically for use in the smaller, more sensitive, more easily irritated airways of infants and children.

Examination of the upper respiratory tract with slim, flexible instruments[6] has added another dimension to airway evaluation in infants and children, particularly in the diagnosis of dynamic abnormalities such as laryngomalacia and vocal cord paralysis. Even so, traditional, rigid, open-tube, direct endoscopy under general anesthesia provides the most reliable and comprehensive assessment and maintains its paramount role. For detailed evaluation of the upper aerodigestive tract during endoscopy, rigid telescopes provide brilliant, magnified, crystal-clear images for diagnostic and teaching purposes, and they facilitate documentation by 35-mm photography[7] and hard copy recording or video recording.

Because laryngeal abnormalities may have coexistent disorders[8] (eg, subglottic stenosis, vascular compression of the trachea, tracheomalacia, gastroesophageal reflux), examination of the pharynx and larynx is usually combined with tracheobronchoscopy and esophagoscopy. Thus, the method of laryngoscopy and anesthesia must allow not only for panendoscopy, but also for the possibility of endoscopic microsurgery or laser surgery.

LARYNGOSCOPES

"Anesthetic" laryngoscopes are used by anesthesiologists to identify the glottic opening for endotracheal intubation, but they are battery-powered, provide poor light intensity, and cannot be suspended. They are appropriate for intubation or for an emergency, but they are unsuitable for thorough evaluation of the laryngopharynx.

"Diagnostic" laryngoscopes can be used by laryngologists[9] for children of any age, even in very small infants weighing less than 1,000 g. Direct endoscopy is normally performed with modern techniques of anesthesia[10,11]; examination with sedation and local anesthesia only is seldom justified.

Laryngoscopes are basically modified open tubes with illumination provided either at the proximal end or at the distal end. Although the pediatric laryngoscopes and ventilating bronchoscopes manufactured by Karl Storz GmbH & Co KG (Tuttlingen, Germany) are used throughout the world, laryngoscopes are available in many other designs. Those favored in North America[12] include the Jackson laryngoscope, the Tucker side-slide laryngoscope, the Holinger newborn laryngoscope, and the Healy subglottiscope. In Japan, the Nagashima equipment may be chosen. The availability of so many pediatric laryn-

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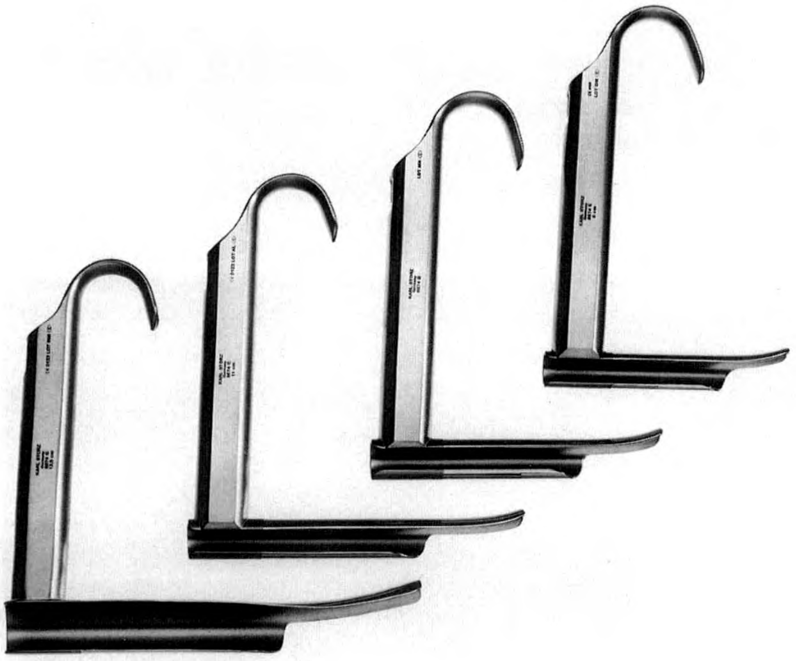

Fig 1. Four sizes of Karl Storz general-purpose laryngoscopes for routine examination.

goscopes has led to confusion with regard to their names and applications.

The choice of laryngoscope for each patient depends on the presenting problem, the area known to need close examination, the procedure to be undertaken, the method of anesthesia, and the personal preference of the laryngologist. It is often helpful to change the size of the laryngoscope or to change from one design to another to improve exposure, eg, in the anterior commissure, posterior glottic space,[13] subglottis, or upper trachea. To manage the many problems that may be encountered, it is necessary to have adequate instrumentation with a variety of different designs and sizes, for patients from very small babies to teenagers.

Certain design features are common to all laryngoscopes, both adult and pediatric. In theory, the requirements for the "ideal" laryngoscope would be many. Such an instrument would be of simple design, be lightweight yet durable, be constructed of good-quality metal, be suitable for sterilization to satisfy appropriate standards, have a comfortable handle with a good overall balance and "feel," have a wide proximal end, provide stereoscopic binocular vision, be atraumatic to teeth, gums, and soft tissue, be anesthesia-compatible, allow uninterrupted administration of anesthetic gases, be suitable for suspension, and, last but most important, be effective in providing the desired exposure. In reality, of course, these criteria are not achievable in any one laryngoscope.

Other design aspects are of particular importance in smaller, more specialized laryngoscopes for pediatric use, eg, modification to assist introduction of an endotracheal tube or bronchoscope (side slot or removable slide), provision for delivery of anesthesia (built-in channel or detachable metal cannula), bright illumination (proximal prism, distal light rod, or detachable all-purpose light clip), capability to expose specific regions (supraglottic, glottic, subglottic, posterior glottic, postcricoid area, or overall view of the laryngopharynx), suitability for microsurgical procedures (removal of cysts or tumors, supraglottoplasty, repair of posterior cleft), and safe use of the laser (papillomas, subglottic hemangioma).

Endoscopic access to the larynx depends on a sufficient depth of anesthesia, the degree of any coexistent micrognathia, the presence and size of teeth, the mobility of the temporomandibular joints, the size and instability of the tongue, the overhang of the epiglottis, the extension of the cervical spine, and the nature and extent of the disorder.

CLASSIFICATION OF PEDIATRIC LARYNGOSCOPES

The laryngoscopes used in the Department of Otolaryngology at the Royal Alexandria Hospital for Children, Sydney, can be divided into 2 groups.

The first group includes those used for general purpose and routine examination. The second group includes those used for special purposes: microlaryngoscopy, microsurgery, laser surgery, and the "difficult" airway or the obstructed airway. Some laryngoscopes are multipurpose.

These laryngoscopes, with photographs to iden-

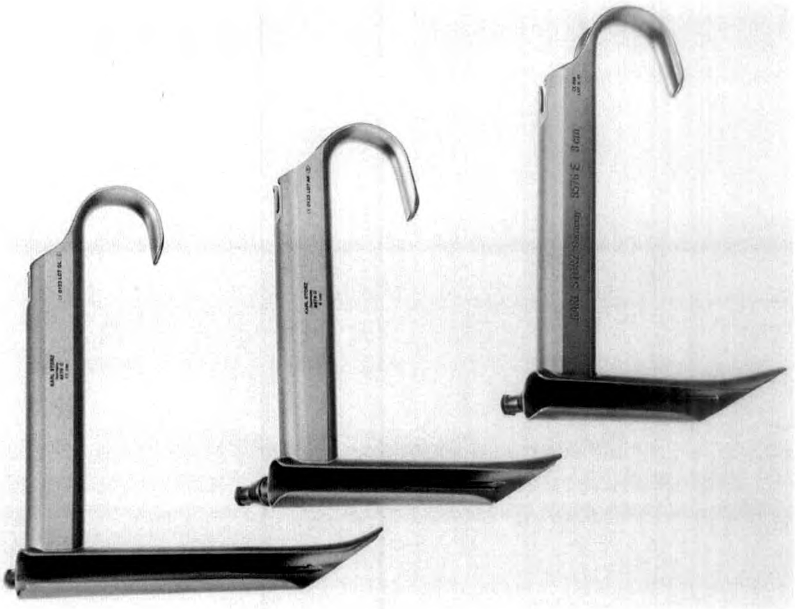

Fig 2. Three sizes of Parsons general-purpose laryngoscopes.

tify them, will be described according to their design, application for diagnosis and treatment, adaptation for anesthesia, lighting, and specifications.

GENERAL-PURPOSE LARYNGOSCOPES

Karl Storz. The Karl Storz instruments are classic, time-tested diagnostic instruments (Fig 1) placed in front of or behind the epiglottis. They have a side slot for introduction of an endotracheal tube or bronchoscope. Anesthesia is administered by insufflation. Lighting is provided by a proximal prism. Usually handheld, the instruments come in 4 sizes — 8574G, 13.5 cm; 8574C, 11 cm; 8574D, 9.5 cm; and 8574E, 8 cm — and can be used for children from newborn to teenage.

Parsons. The distal tip of the Parsons laryngoscope (Fig 2) may be placed either in front of or behind the epiglottis, near the anterior commissure. There is a side opening for introduction of an endotracheal tube or bronchoscope. A metal cannula is fixed in the left-hand side for continuous insufflation of oxygen or gases for anesthesia. Lighting is by proximal prism. The laryngoscopes are usually hand-held, and come in 3 sizes — 8576C, 11 cm; 8576D, 9 cm; and 8576E, 8 cm — for ages newborn to 5 years.

SPECIAL-PURPOSE LARYNGOSCOPES

Benjamin-Lindholm. The Benjamin-Lindholm laryngoscopes provide an ideal overview for diagnosis and operative surgery (Fig 3). The wide diameter gives a panoramic, binocular perspective of the laryngopharynx and endolarynx for naked-eye, tele-

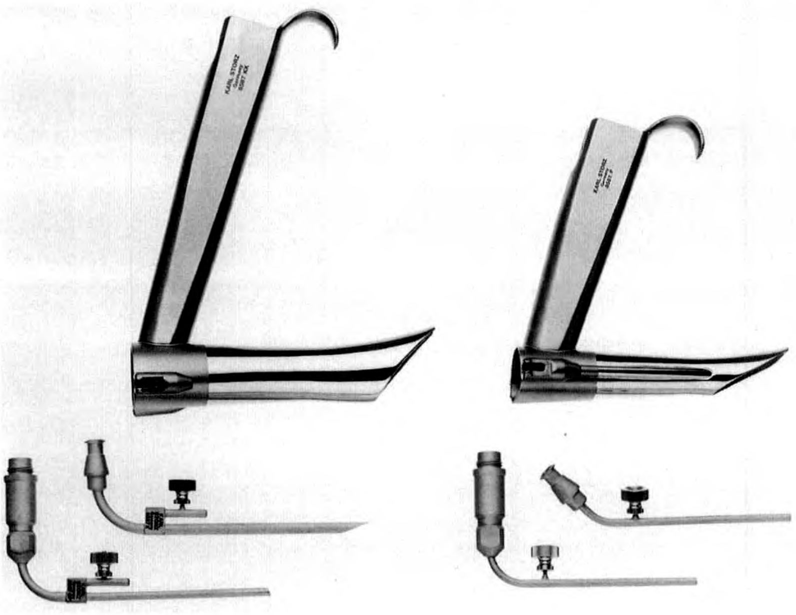

Fig 3. Two sizes of Benjamin-Lindholm diagnostic and operative laryngoscopes (middle size not shown).

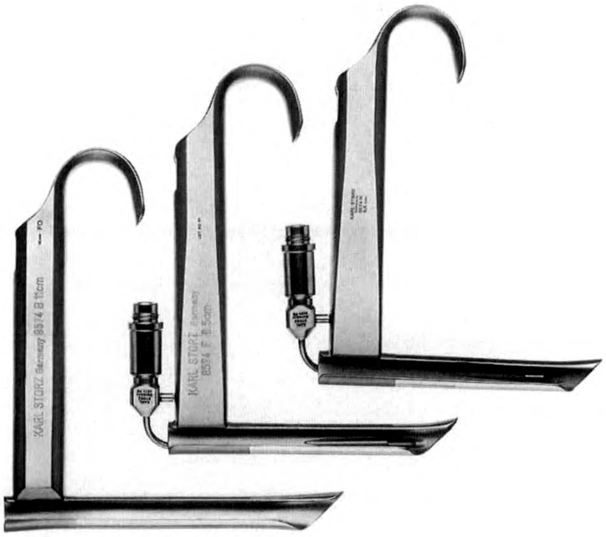
KARL STORZ Germany 8574 B 11cm
KARL STORZ Germany 8574 F 9.5cm

Fig 4. Three sizes of Holinger-Benjamin laryngoscopes for difficult laryngoscopy and intubation in infants.

scopic, or microscopic examination. The broad distal tip is positioned in the midline at the base of the tongue in the valleculae, against the glossoepiglottic fold to elevate the epiglottis out of the field of view. The comprehensive exposure obtained from distension of the pharynx by the wide body facilitates endolaryngeal operations. The laryngoscopes are ideal for suspension and ideal for documentation, and have a metal cannula for anesthesia. Lighting is by proximal light rod or detachable clip. There are 3 sizes — 8587KK, 11 cm; 8574N, 11 cm; and 8587P, 9.5 cm — for all ages from babies to teenagers.

Holinger-Benjamin. Small-diameter, monocular diagnostic laryngoscopes (Fig 4) designed to expose difficult areas such as the anterior commissure, posterior glottic space, and subglottis, the Holinger-Benjamin laryngoscopes have a side slot that is essential for intubation of the "difficult" airway, eg, in Pierre Robin syndrome, in which intubation may be difficult or impossible with other laryngoscopes. They are not used for suspension, and come in 3 sizes — 8574B,11 cm; 8574F, 9.5 cm; and 8574N, 9.5 cm (slim model) — for neonates and low–birth weight infants.

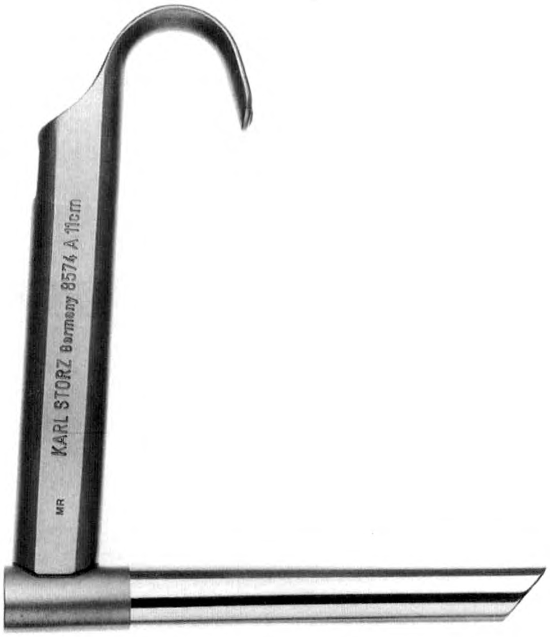

Fig 5. One size of Kleinsasser pediatric diagnostic and operating laryngoscope (other size not shown).

Kleinsasser. The Kleinsasser laryngoscopes are well-known standard, diagnostic and operating laryngoscopes (Fig 5) for visualization of the vocal cords. They are suitable for suspension microlaryngoscopy and surgical treatment (8574A, 11 cm, for infants).

Benjamin Operating Laryngoscopes. The Benjamin operating laryngoscopes are for microlaryngoscopy and surgical or laser treatment (Fig 6). They

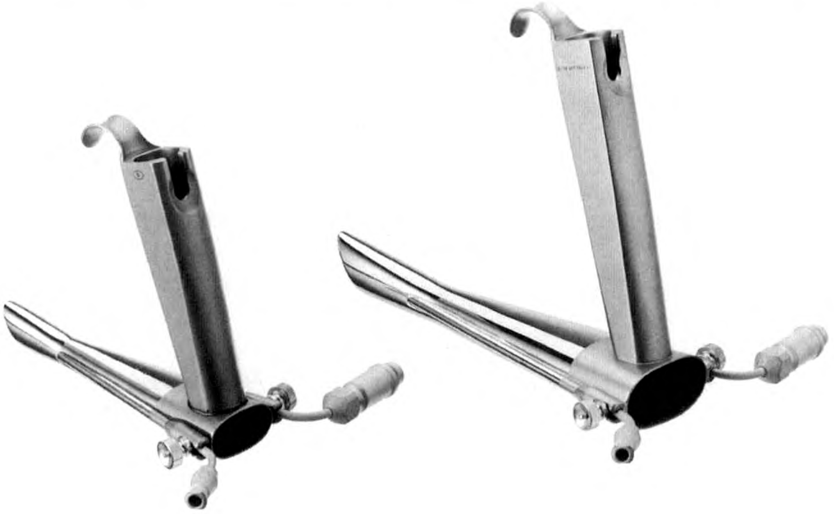

Fig 6. Two sizes of Benjamin operating laryngoscopes for microsurgery and laser surgery.

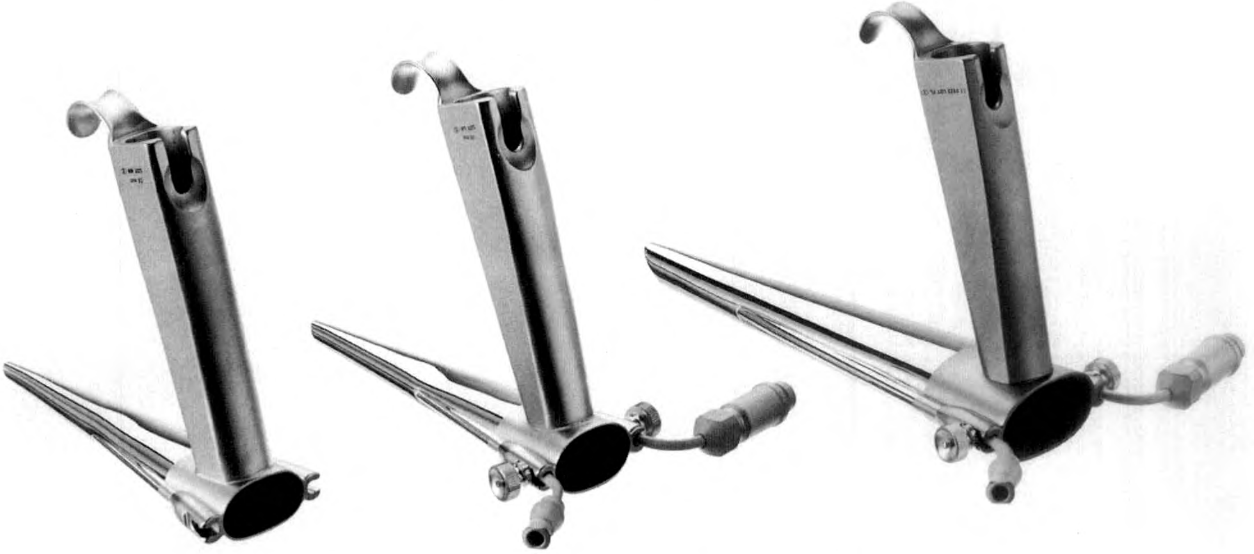

Fig 7. Three sizes of Benjamin diagnostic and operating subglottiscopes.

have a wide proximal end for binocular viewing, easy insertion of instruments, and safe use of the laser. There is a modification with a side slot. They are designed for suspension and have a recessed metal cannula for anesthesia. Lighting is by a detachable proximal clip or a light rod. They come in 2 sizes — 8574J, 15 cm, and 8574JB, 11.5 cm — and can be used for all ages.

Benjamin Subglottiscopes. The Benjamin subglottiscopes have a wide proximal end (Fig 7) for binocular diagnostic inspection, and for surgical and laser treatment in the subglottis and upper trachea by separation of the vocal cords with the narrow distal end. They are suitable for suspension and have a metal cannula for anesthesia. Lighting is by a distal light rod. There are 3 sizes: 8574R, 14 cm (internal distal diameter, 7.0 mm); 8574RA, 11 cm (internal distal diameter, 4.7 mm); and 8574RB, 11 cm (internal distal diameter, 3.5 mm). The larger instrument is for children, and the 2 smaller ones are for infants.

Suspension for all of these laryngoscopes is with the Benjamin-Parsons pediatric laryngoscope holder. It allows use of both hands, provides an unchanging view by maintaining the laryngoscope firmly in the airway, and allows diagnostic examination of the tracheobronchial tree with rigid telescopes while the suspension laryngoscope remains in place. In this way, using a slim telescope introduced through a laryngoscope is less traumatic than using a bronchoscope and does not distort the tracheobronchial anatomy.

ANESTHESIA

Laryngoscopy is performed in a fully equipped operating room with facilities for monitoring heart rate, electrocardiogram, respiratory function, and pulse oximetry. A selection of equipment for resuscitation and a full range of laryngoscopes and ventilating bronchoscopes must be available.

The technique of general anesthesia is decided on after consultation between the anesthesiologist and the endoscopist. For infants and younger children, we prefer the spontaneous respiration inhalational technique.[10,11] The routine use of supplemental topical local anesthesia is recommended. To provide the best conditions for older children (over the age of approximately 12 years), we use a muscle relaxant technique, with intermittent jet ventilation, similar to that used in adults.[10]

LARYNGOSCOPY

Indirect flexible laryngoscopy for assessment of vocal fold motion is often performed with the patient awake, before the induction of general anesthesia. For initial direct examination, a general-purpose laryngoscope of suitable size is introduced for naked-eye assessment. External finger pressure on the anterior part of the neck may be helpful, and manipulation of the larynx with gentle counterpressure from the distal beak of the laryngoscope will better display the anterior commissure and subglottic larynx.[9] Suspension and evaluation with 0°, 30°, and, if necessary, 70° telescopes builds up a "3-dimensional" concept of the disorder before microlaryngoscopy and endolaryngeal surgery is undertaken. Thus, systematic examination of the laryngopharynx is performed in 3 stages, as follows.

HANDHELD LARYNGOSCOPY

Preliminary examination of the oropharynx and larynx is undertaken with a handheld laryngoscope.

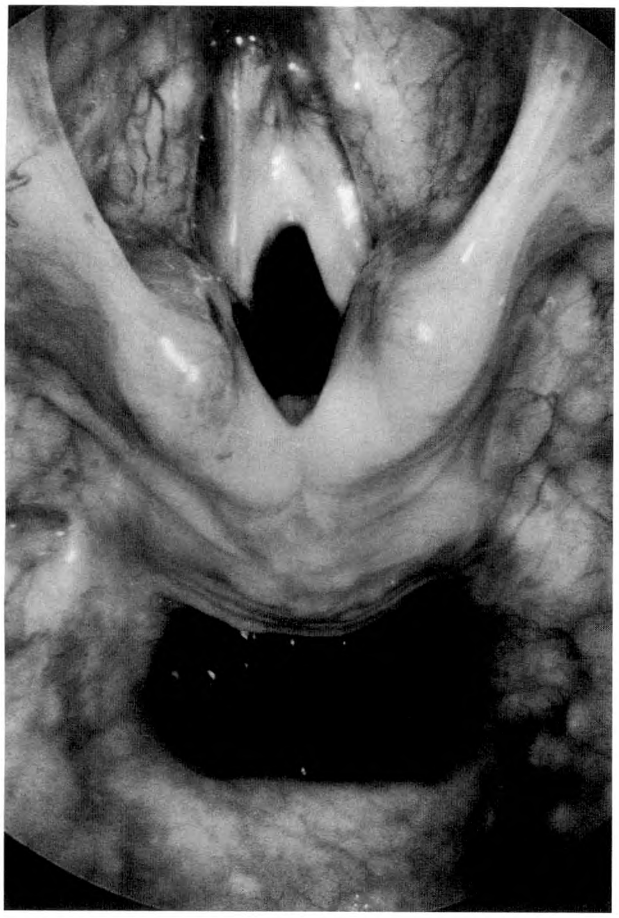

Fig 8. Exposure of hypopharynx, postcricoid region, and proximal esophagus with "laryngeal lift." Telescopes are used for detailed examination. Note congenital anterior glottic web.

The airway for anesthesia is secured, and the site of the disorder is localized with the naked eye and/or with a telescope. A decision can be made as to whether to continue with the chosen laryngoscope or to change to another size or design. Optimal exposure is a basic principle.

SUSPENSION LARYNGOSCOPY

An unchanging, stable view is provided by suspension laryngoscopy. Both hands are available for precise evaluation with telescopes, instrumentation, and documentation.

MICROLARYNGOSCOPY

Microlaryngeal surgery or laser surgery with the operating microscope requires a laryngoscope with a wide proximal opening to enhance binocular viewing for easier, unobstructed introduction of instruments and for safe use of the laser.

With the larynx in suspension, first rigid rod lens telescopes (of different lengths, diameters, and optical viewing angles) and then the microscope are used for detailed examination, teaching, and documentation. Whereas telescopes give flexibility of visualization and documentation, with the microscope, observation and photography are more or less confined to the endolarynx.

Pressure exerted by the hand of the surgeon or an assistant on the neck with counterpressure from the beak of the laryngoscope improves exposure as much as the anatomy of the patient will permit. Gentle downward and outward pressure with a beaded suction tip or blunt probe just lateral to the edge of the membranous vocal fold will "roll" it to expose its subglottic surface. Lateral retraction of the ventricular fold with a sucker and external neck pressure to rotate the larynx to the other side makes it possible to inspect most of the laryngeal ventricle, if not by direct view, then with telescopes. The straight-ahead and angled telescopes are used for other difficult areas, such as the valleculae, pyriform fossae, anterior commissure, posterior glottic space, and subglottis. The postcricoid region can be brought into view (Fig 8) with the "laryngeal lift" maneuver by grasping and elevating the thyroid cartilage through the skin of the neck. The membranous vocal folds are palpated for pliability, and the mobility of the cricoarytenoid joints is tested.

Thus, by use of telescopes and the microscope, a comprehensive idea of the nature and extent of any abnormality is built up before any endolaryngeal surgery is commenced. Laryngoscope selection will be influenced by the demands of the particular problem, for instance, to meet the needs of a preliminary diagnostic evaluation (extent of laryngeal papillomas), an overall panoramic view (diagnosis of laryngomalacia), endoscopy in a small infant (assessment of intubation trauma), inspection of the subglottic area (subglottic stenosis), posterior glottic evaluation (congenital cleft), management of a difficult airway (micrognathia and Pierre Robin syndrome), emergency restoration of an obstructed airway (acute supraglottitis), or endolaryngeal surgery (supraglottoplasty).

A knowledge of the design advantages of the various pediatric laryngoscopes will influence the selection for each patient.

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NINTH INTERNATIONAL FACIAL NERVE SYMPOSIUM

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CHROMOSOMAL ABNORMALITIES IN IDIOPATHIC CONGENITAL BILATERAL VOCAL CORD PARALYSIS

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Idiopathic congenital bilateral vocal cord paralysis (BVCP) can occur as an isolated abnormality or as part of a multisystem disorder. The chromosomal abnormalities found in 4 children who presented with idiopathic congenital BVCP in association with other congenital abnormalities between January 1991 and April 1999 are described. Three children had abductor paralysis, and tracheostomy was required in 1. The fourth child had adductor paralysis and required a feeding gastrostomy for management of aspiration. There was no clinically significant improvement in vocal cord function observed in any child. Idiopathic congenital BVCP, when associated with other congenital disorders, may occur as a consequence of an underlying chromosomal abnormality, and chromosome studies should be performed in this group of children. Similarly, the possibility of vocal cord dysfunction should be considered in children who are found to have chromosomal abnormalities. A chromosomal abnormality appears to preclude the possibility of early spontaneous improvement in vocal cord function.

KEY WORDS — chromosomal abnormality, congenital stridor, karyotype, vocal cord paralysis.

INTRODUCTION

Congenital bilateral vocal cord paralysis (BVCP) is a neurologic or a neuromuscular disorder that affects the intrinsic laryngeal muscles, typically involving laryngeal abduction and presenting with stridor. Endotracheal intubation at birth with early tracheostomy may be necessary. Congenital BVCP also, rarely, presents as adductor paralysis.[1] It may occur as an isolated abnormality or in the context of associated defects. It appears sporadically, and rarely, presents as a familial trait.

Congenital BVCP may be secondary to such extrinsic agents as birth trauma or asphyxia, or may be the consequence of a structural or functional nervous system defect. In a significant number of cases, no cause is found.[1]

Idiopathic congenital BVCP can be broadly classified into isolated cases and those associated with other defects.[1] Presumably, there is heterogeneity of cause within these groupings. Holinger et al[2] reviewed 114 children with congenital BVCP, and could find no cause in 71. There were associated congenital defects in 32 of these children, including neurologic, cardiac, and laryngeal abnormalities, and 3 of these children had chromosomal abnormalities. In a smaller pediatric series of 19 cases of congenital vocal cord paralysis, Emery and Fearon[3] found 10 children in whom the cause was unknown, and 5 of these had additional abnormalities. Two had an abnormal karyotype. With so few reports of chromosomal findings in congenital BVCP associated with other abnormalities, we decided to review our experience, and describe here 4 cases.

METHODS

A retrospective chart review was carried out to identify children presenting to the Royal Children's Hospital, Melbourne, Australia, with symptomatic BVCP (either abductor or adductor) that was congenital in origin and associated with other congenital abnormalities. Only those patients in whom no pathogenic mechanism could be identified were included. In particular, there was no history of birth trauma or birth asphyxia, no computed tomographic or magnetic resonance imaging evidence of a major structural central nervous system abnormality, no clinical suspicion of a neuromuscular disorder, and no structural laryngeal abnormality found at microlaryngoscopy or bronchoscopy. The results of karyotyping were recorded.

RESULTS

Patient details and results of chromosome analysis of the 4 patients identified are listed in the Table. Three presented with abductor BVCP and associated stridor, 1 of whom required a tracheotomy. The fourth patient presented with adductor BVCP and required a feeding gastrostomy for management of aspiration. The patients all displayed different karyotype abnormalities.

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DETAILS OF FOUR KARYOTYPICALLY NORMAL PATIENTS WITH IDIOPATHIC CONGENITAL BILATERAL VOCAL CORD PARALYSIS ASSOCIATED WITH OTHER CONGENITAL ABNORMALITIES

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*
Type of paralysis	Abductor	Abductor	Abductor	Adductor
Major symptom	Stridor	Stridor	Stridor	Aspiration
Intervention	Tracheostomy	None	None	Gastrostomy tube
Associated abnormalities	Developmental delay	Developmental delay, cystic renal dysplasia	Typical Down syndrome	22q Deletion syndrome with unilateral choanal atresia, submucous cleft palate, atrioseptal defect
Chromosomal abnormality	Rearrangement of chromosomes 14 and 18, with partial deletion, and paracentric inversion: 46,XX,inv(12) (q15; q21.2), rea (14;18) (q12;q12.1)	Balanced 5/11 translocation: t(5;11) (q23.1;q14.2)	Trisomy 21	Interstitial deletion of chromosome 22: 46,XY,del (22) (q11.22; q12.2)
Age at follow-up	3 y	3 y	8 wk	8 y
Progress	Unchanged	Unchanged	Death due to respiratory failure at 8 weeks of age	Unchanged

DISCUSSION

We report on 4 patients with idiopathic congenital BVCP associated with other congenital abnormalities who had an abnormal karyotype. The chromosomal basis was of a different category in each. In case 1, there was a deletion of either 14q12 or 18q12.1, due to a de novo 14;18 rearrangement. In addition, there was a de novo inversion of chromosome 12; however, further studies to delineate the imbalance more precisely could not be done, as the family lived overseas. In the second case, a de novo apparently balanced translocation was identified. The break points were at band q23.1 on chromosome 5 and at band q14.2 on chromosome 11. The third and fourth patients had well-known chromosomal syndromes: standard trisomy 21, and 22q deletion syndrome.

There have been 2 reports in the literature describing chromosomal abnormalities in a series of children with congenital vocal cord paralysis. In the series reported by Holinger et al,[2] there were 32 cases of BVCP with associated malformations, which included 3 with a karyotypic defect. Two patients had trisomy 21, both with concomitant cardiac defects. One child, with laryngomalacia, hypospadias, and bilateral hip dysplasia, had a D group short arm variant, but current knowledge reveals it likely to have been simply a normal variant chromosome. Emery and Fearon[3] reported 1 child with BVCP and another with unilateral vocal cord paralysis with translocation of part of chromosome 14, but no further karyotype detail was provided. Single cases of stridor without documentation of vocal cord function in association with certain abnormal karyotypes have been reported. These have included deletions of 10p,[4] 18p, and 18q[5] and trisomy 19 mosaicism.[6] On the basis of the limited information in the literature and our own cases, it appears that only duplication of chromosome 21 has been associated with more than 1 case of BVCP. Because Down syndrome is so common, its association with only 3 documented cases is not, in fact, very pressing grounds for suggesting a strong causal link.

There are no prognostic indicators to suggest the likelihood of spontaneous recovery in idiopathic congenital BVCP. In particular, laryngeal electromyography has not been shown to be of prognostic value in this condition.[1] None of the 4 children in our study showed evidence of clinically significant improvement in vocal cord function after a prolonged period of follow-up. This finding suggests that BVCP in the presence of a chromosomal abnormality is unlikely to show spontaneous resolution.

Children with idiopathic congenital BVCP associated with other congenital abnormalities should be referred to a geneticist for clinical evaluation and chromosome analysis. Similarly, the possibility of vocal cord paralysis should be considered in children with chromosomal abnormalities who have symptoms indicative of laryngeal dysfunction.

Structural chromosomal rearrangements have the potential to point the way to the discovery of causative genes, if a break point should happen to disrupt a particular locus, and a number of successful examples of this approach are on record. Rare cases of familial BVCP[7] confirm the existence of single genes that can cause BVCP, probably by some effect upon the neural substrate in the brain stem. Thus, the break points documented above (5q23.1, 11q14.2, 14q12, and 18q12.1) offer candidacy as the location(s) of such a gene or genes.

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COSMETIC FACIAL SURGERY

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Ann Otol Rhinol Laryngol 110:2001

EVALUATION OF VOICE QUALITY IN ADDUCTOR SPASMODIC DYSPHONIA BEFORE AND AFTER BOTULINUM TOXIN TREATMENT

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In this prospective study, the efficacy of botulinum toxin (Botox) injections in patients with adductor spasmodic dysphonia (AdSD) was assessed by 3 different modalities: perceptual and acoustic analyses and subjective self-assessment. This was done by comparing AdSD patients' pretreatment and posttreatment values and comparing these values with those of normal control speakers. In contrast to most other studies, the posttreatment status was defined as the optimal voice quality as judged by the patient. The aim of the study was to assess to what extent Botox injections actually improve voice quality and function. The AdSD subjects rated a significantly improved voice quality and function after Botox treatment. However, the results were never within normal limits. Perceptually, the characteristic and severely impaired AdSD voice improved, but another "type" of pathological voice was detected after Botox treatment. Acoustic analyses demonstrated a significant improvement, as well. Nevertheless, the "optimally" treated AdSD voice still remained significantly deviant as compared to normal voice production. Currently, Botox injection is the therapy of first choice for AdSD. Although significant improvement could be measured in our study perceptually, acoustically, and subjectively, the optimal voice that was achieved never fully matched normal voice quality or function.

KEY WORDS — acoustic analysis, GRBAS rating scale, perceptual assessment, spasmodic dysphonia, voice quality.

INTRODUCTION

Adductor spasmodic dysphonia (AdSD) is an uncommon and poorly understood voice disorder that is probably due to laryngeal dystonia. The vocal symptoms are characterized by difficulty with voice initiation, a strain-strangled, effortful phonation with voice breaks, and glottal fry. The current treatment of choice for AdSD is botulinum toxin (Botox) injections in the vocal folds.[1] Most papers dealing with AdSD and Botox treatment conclude that these patients, as a group, have benefited.

Several modalities have been used to evaluate the success of Botox treatment: patient's self-evaluation of voice quality and performance, perceptual voice ratings, acoustic analysis, flexible videolaryngoscopy, aerodynamic measurements, and neurophysiological and psychosocial testing.[2] For all of these modalities, significant improvement was found. Efficacy was evaluated either by comparing patients' pretreatment and posttreatment conditions or by comparing the pretreatment or posttreatment values with those of normal controls. Available studies in the literature tend to concentrate on only 1 modality in appraising the effectiveness of this therapy. For example, some studies report improvement on the basis of patient self-rating scales, while others only rely on aerodynamic or acoustic measurements. Moreover, the frequency of recordings, and moments in time at which the voice was recorded after Botox injection, varied considerably among clinical investigators. Most studies assessed the voice at some arbitrary interval (eg, 2, 4, or 6 weeks) after injection. What is lacking is research focusing on "optimal" voice quality and performance after Botox treatment and in which different modalities are combined.

Therefore, the aim of the present study was to assess to what extent voice quality and performance actually improve through injection with Botox. This was done by comparing patients' pretreatment voice with the optimal voice (as judged by the patient), as well as comparing both pretreatment and optimal voices with normal control voices. The most frequently used testing modalities were applied: perceptual ratings, (temporal) acoustic analysis, and self-assessment scores.

PATIENTS AND METHODS

SUBJECTS

Two groups participated in this study. One group consisted of 46 patients with AdSD. Diagnosis of AdSD was made independently by a speech pathologist and 2 otorhinolaryngologists.[3] Fourteen of these

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TABLE 1. VOICE QUALITY PARAMETERS AND DEFINITIONS OF EXTENDED GRBAS SYSTEM

GRBAS	
Grade	Overall degree of impairment
Roughness	Low-frequency aperiodic noise; random fluctuations of glottal pulse
Breathiness	Audible turbulent noise generated at glottis
Asthenia	Weak, powerless voice; no carrying power
Strain	Excessive tension in vocal cords
Extension	
Aphonia	Voice lacking in phonation; whispering voice
Diplophonia	Two different pitches that can be simultaneously perceived
Staccato	Abrupt voice initiation and arrest; irregular interruption of voice
Tremor	Rapidly occurring fluctuations in pitch and/or loudness; quavering voice
Falsetto	Mode of phonation
Vocal fry	Low-frequency periodic vibration

Reprinted from Langeveld et al.[5]

subjects were men with a mean age of 47 years (range, 32 to 72 years), and 32 were women with a mean age of 49 years (range, 19 to 82 years). The second (control) group consisted of 46 normal subjects with no known speech, hearing, or neurologic disorders. The 2 groups were matched for age (±1 year) and sex.

TIME SCHEDULE OF RECORDINGS

This study was part of a larger prospective clinical trial in which different procedures of Botox injection were compared in AdSD patients. All 46 patients underwent unilateral and bilateral injections. During the first procedure, 5 units (1.25 U/0.1 mL) of Botox was injected in the left thyroarytenoid muscle only. The second treatment was performed after the voice quality had returned to the preinjection level (as established by the patient and by audio and video registrations). During this procedure, both thyroarytenoid muscles were injected, each with 2.5 units (1.25 U/0.1 mL) of Botox. Under electromyographic guidance, a monopolar 27-gauge 30-mm Teflon-coated needle was inserted percutaneously, through the cricothyroid membrane and into the left thyroarytenoid muscle, during the first procedure, or into both thyroarytenoid muscles, during the second procedure.

The choice for the next treatment depended on the patient's preference. The dose was adjusted by titrating efficacy and side effects. The end point of the study was the moment at which the patient experienced an optimal voice quality and performance. This was obtained after 3 Botox injections in 26 patients, after 4 injections in 18 patients, and after 5 injections in 2 patients. Thirty-five patients were most satisfied after a bilateral injection, and 11 after a unilateral injection. The dosages varied: 5, 7.5, and 10 units in 20, 23, and 3 patients, respectively.

The moment the patients entered the study (pretreatment), they were asked to complete a self-rating questionnaire for judgment of the voice. At that time, recordings were made for perceptual and acoustic evaluation of the voice quality. The moment the patients judged their voices to be optimal, all tests and recordings were repeated. Recordings of the normal control group were obtained over a period of 4 weeks. The following within- and between-group comparisons could therefore be made: 1) within the AdSD group, differences between pretreatment and posttreatment voices; 2) between groups, differences between the AdSD pretreatment group and normal controls; and 3) between groups, differences between the AdSD posttreatment group and normal controls.

PERCEPTUAL ANALYSIS

The most widely used perceptual rating system is the GRBAS scale, based on the work of Isshiki et al.[4] However, with this system, not all audible features of AdSD can be described. Therefore, in a previous study,[5] we extended the GRBAS system with 6 relevant parameters. It was demonstrated that the "extended" GRBAS system could be used for accurate and reproducible perceptual characterization of AdSD. Since symptoms of AdSD are markedly manifest during conversational speech, spontaneous speech was recorded for perceptual evaluation (see section on acoustic analysis data collection, below). The voice quality parameters and their definitions are listed in Table 1.[5] The ratings were performed on visual analog scales. On the score form, each parameter was scored on a continuous horizontal line of 10 cm, from normal on the left side to extremely pathological on the right side. The distance in centimeters from the left side determined the score. All voice samples were evaluated and scored by 3 experienced observers who were familiar with both spasmodic dysphonia and the extended GRBAS system. In those instances in which the judges rated voice quality differently, a consensus was reached through reevaluation and discussion.

SUBJECTIVE VOICE EVALUATION

For the subjective evaluation of vocal function, a questionnaire was designed that consisted of several aspects of voice quality and function. These included the intelligibility of speech (conversational speech, during a party, in a public gathering), fatigue (tiring and shortness of breath), loudness, mode of phonation (fluency and strain), and known side effects of Botox treatment, such as hoarseness or a breathy voice and choking on fluids. The ratings were performed on visual analog scales (10 cm) from severe or poor on the left side to normal or good on the right side. The distance in centimeters from the left side determined the score. During the first week after the injection, rating was on a daily basis. Thereafter, the rating was weekly for at least 3 months.

ACOUSTIC ANALYSIS

Data Collection. All the speech material was recorded in a sound-treated room, with a Kennet ET 2100 electret microphone, a Sony Hi-8 videocassette camera recorder, and a JVC HiFi stereo videocassette recorder. The recording level was adjusted for each subject to optimize the signal-to-noise ratio and was then kept constant for the remainder of the recording. The mouth-to-microphone distance was kept at approximately 30 cm.

The subjects were asked to produce a number of sustained vowels /a/ (as in the Dutch word *aap*) at a comfortable pitch and loudness level. They also had to repeat a standard Dutch sentence (*Adam at altijd een appel op*; "Adam always ate an apple") at a normal conversational level. For each subject, the vowel and sentence that sounded most like the subject's habitual voice were analog-to-digital converted (sampling frequency, 44.1 kHz) and digitally filtered with a 16th-order bandpass Butterworth filter (80 Hz to 15 kHz). The signal was stored on a computer system and used for subsequent acoustic analyses. A total of 92 vowels and sentences (46 AdSD patients and 46 matched controls) were therefore stored in separate files.

All acoustic analyses were done with PRAAT, a software package for processing speech signals.[6] A number of acoustic parameters were evaluated to give a more complete description of the voice and speech quality of the AdSD patients.

Temporal Acoustics. As the involuntary disruption of phonation seen in AdSD also interferes with fluent speech, the following temporal aspects of speech production were evaluated for the standard sentence.[7] The total speaking time (TST) was the total duration of a sentence measured from the onset of the first word to the offset of the last word. The onset and offset of acoustic energy were determined perceptually and through visual assessment of oscillographic and spectrographic information. The total interword interval time (TIIT) was the total of the durations of all interword silences exceeding 10 ms. The total articulation time (TAT) was the total duration of the sentence minus the interword interval time (TST minus TIIT).

Vowel Onset Times. Patients with AdSD are known to have particular difficulty with the initiation of speech.[8] It is, however, not known whether the time it takes for the vocal folds to enter a stable mode of vibration is affected by the laryngeal spasms seen in these patients. It is, furthermore, unclear whether vowel onset times (VOTs) are task-related — in other words, whether it is more difficult to initiate a sustained vowel in isolation than to initiate a vowel in running speech. To obtain some insight into VOTs, we manually measured the durations of vowel onsets (of both the sustained vowel and the first /a/ in the sentence: *Adam at altijd een appel op*; "Adam always ate an apple"). Vowel onset was defined as the duration from the beginning of the vowel to the point at which the signal becomes regular in terms of periodicity.[9] Within- and between-group comparisons were made.

Proportion of Aperiodicity and Silence per Vowel. Sapienza et al[10] found aperiodicity, frequency shifts, and phonatory breaks to be useful in classifying phonatory behavior associated with AdSD. The (autocorrelation) algorithm for periodicity detection was used to determine episodes of aperiodicity and silence within a sustained vowel.[11] This algorithm is a short-term analysis method; therefore, the analysis is performed for small segments (frames) that are taken from the (voice) signal in steps of 0.01 second. The appropriate arguments of the pitch detection algorithm were adjusted so that the number of aperiodic frames and silent frames per vowel could be assessed.

The proportion of aperiodicity was determined as follows. If a time frame did not contain an autocorrelation peak above 0.8 relative to the maximum possible autocorrelation, the frame was classified as unvoiced (aperiodic). This value was chosen because it corresponded with aperiodicity as judged through visual inspection of the oscillogram (nonrepetitiveness of cycles) and perceptual judgment of the voice quality. The number of unvoiced frames was then divided by the total number of frames (total duration of vowel), resulting in a value between 0 and 1.

The proportion of silence was determined as follows. Frames that did not contain amplitudes above 0.1 (relative to the global maximum amplitude) were considered silent. The number of silent frames was then divided by the total number of frames, resulting in a proportional value between 0 and 1. This was considered as representative of the proportion of voice breaks.

Instability of Phonation. Laryngeal instability associated with AdSD has a profound influence on the ability to maintain steady phonation, as well as on the voice quality. Several acoustic parameters have been employed as objective measurements of severity, of which those parameters dealing with the stability of phonation seem to be the most useful, eg, fundamental frequency standard deviation (F0 SD).[8,12-15]

TABLE 2. PARAMETERS OF EXTENDED GRBAS SYSTEM BEFORE AND AFTER BOTOX TREATMENT

Parameter	*Pretreatment*	*Posttreatment*	*p**
Grade	6.3 ± 1.7	0.7 ± 0.9	<.001
Roughness	0.2 ± 0.7	0.4 ± 0.9	.14
Breathiness	1.0 ± 1.7	0.4 ± 0.7	.21
Asthenia	3.3 ± 1.7	0.5 ± 0.8	<.001
Strain	5.5 ± 2.1	0.2 ± 0.4	<.001
Aphonia	0.6 ± 1.8	0.0 ± 0.0	.018
Diplophonia	0.0 ± 0.0	0.0 ± 0.0	
Staccato	3.1 ± 2.8	0.1 ± 0.3	<.001
Tremor	0.6 ± 1.1	0.2 ± 0.6	.028
Falsetto	0.1 ± 0.3	0.0 ± 0.0	.18
Vocal fry	2.6 ± 3.4	0.2 ± 0.8	<.001

Data are mean ± SD; 0 represents "normal" and 10 "extremely pathological" voice quality.

*Wilcoxon matched-pairs signed ranks test.

TABLE 3. CORRELATIONS BETWEEN OVERALL PERCEPTUAL GRADE AND PARAMETERS OF EXTENDED GRBAS SYSTEM

	Overall Grade	
Parameter	*Pretreatment*	*Posttreatment*
Roughness	.08	.72*
Breathiness	−.00	.51*
Asthenia	.30†	.56*
Strain	.92*	.40§
Aphonia	.20	
Staccato	.36†	.25
Tremor	−.15	.15
Falsetto	.12	
Vocal fry	.18	−.03

*$p < .001$; †$p < .05$; §$p < .01$.

Therefore, the following acoustic analyses were performed on 2 separate segments of the sustained vowel: the initial 2 seconds (including the vowel onset) and a 2-second segment extracted from the middle, stable part of the sustained vowel. All vowel segments were provided with a linear ramped offset of 10 ms to avoid abrupt audible signal transitions. We analyzed 1) F_0 SD: standard deviation of the F_0 in hertz (F_0 was determined with an autocorrelation pitch detection algorithm[11]); 2) MAS: mean absolute slope in hertz per second; 3) PNR: periodicity-to-noise ratio in decibels, representing the periodicity of the signal; 4) PNR SD: standard deviation of the PNR in decibels; 5) intensity SD: standard deviation of the intensity in decibels; and 6) intensity range: difference in decibels between the minimum and maximum intensities within a segment.

STATISTICAL ANALYSIS

The perceptual voice characteristics, subjective voice assessment scores, and acoustic parameters were summarized with means and SDs when the distribution was approximately normal. In case of extremely skewed distributions, the median and the interquartile range (IQR) were used. Treatment efficacy was assessed by comparing pretreatment and posttreatment scores by the Wilcoxon matched-pairs signed ranks test. Temporal and acoustic parameters were compared with the matched-control group (pretreatment and posttreatment) by the Mann-Whitney test. Throughout this study, a p value of .05 or less was considered statistically significant.

RESULTS

The changes in the perceptual voice quality parameters are given in Table 2. The average pretreatment score of the overall grade of the voice quality was 6.3 (SD, 1.7), and the posttreatment score was 0.7 (SD, 0.9; $p < .001$). The predominant characteristics of the pretreatment voice quality were strain (5.5), asthenia (3.3), staccato (3.1), and vocal fry (2.6). After treatment, all 4 predominant characteristics diminished significantly. However, on average, the voices were not judged as normal (overall grade, 0.7). Some breathiness and asthenia remained. Moreover, roughness had increased after treatment.

Table 3 shows the correlations between the overall perceptual grade and the other voice quality parameters of the extended GRBAS system. Before Botox treatment, the overall grade was predominantly determined by strain (correlation, $r = .92$). Staccato and asthenia correlated weakly but significantly with the overall grade ($r = .36$ and $r = .30$, respectively). Only strain and staccato appeared as independent determinants of the overall perceptual grade. After treatment, the most important determinants of the overall grade were roughness ($r = .72$), asthenia ($r = .56$), and breathiness ($r = .51$). Remarkably, strain, the most predominant perceptual characteristic before treatment, still correlated moderately with the overall grade ($r = .40$) after treatment.

Correspondingly, the self-assessment scores concerning voice quality and performance all improved significantly after treatment (Table 4). However, on average, the patients did not judge their voices as normal, especially with respect to loudness (score, 6.7), intelligibility of the voice in public gatherings (6.8), and hoarseness (7.6).

Table 5 provides the results of the acoustic analyses. Fluency of speaking improved significantly as reflected by the reduced total speaking ($p < .001$), interword interval ($p < .001$), and articulation ($p < .001$) times. However, when pretreatment and posttreatment values were compared with values of normal controls, only the interword interval time had improved to such an extent (posttreatment) that it no longer differed significantly from normal control val-

TABLE 4. SELF-ASSESSMENT SCALES BEFORE AND AFTER BOTOX TREATMENT

	Pretreatment	*Posttreatment*	*p**
Intelligibility			
Conversation	3.7 ± 2.7	9.0 ± 1.0	<.001
Party	1.7 ± 1.8	7.6 ± 2.5	<.001
Gathering	1.3 ± 1.7	6.8 ± 2.9	<.001
Tiring	2.3 ± 2.4	8.8 ± 1.5	<.001
Loudness	3.2 ± 2.8	6.7 ± 2.4	<.001
Fluency	2.9 ± 2.1	8.7 ± 1.2	<.001
Strain	2.3 ± 2.3	8.9 ± 1.3	<.001
Shortness of breath	4.4 ± 3.2	8.7 ± 1.6	<.001
Hoarseness	3.6 ± 3.2	7.6 ± 2.5	<.001
Choking	8.4 ± 2.3	9.0 ± 1.8	.089

Data are mean ± SD; 0 represents "severe or poor" and 10 "normal or good" voice quality or performance.

*Wilcoxon matched-pairs signed ranks test.

ues. Remarkably, the VOT measured in both sustained and running speech did not show a significant improvement. On the other hand, the proportions of aperiodicity and silence (voice breaks) were significantly reduced after treatment. Again, when these values are compared to those of normal controls, the differences remain significant.

In comparing the initial 2-second segment of the sustained vowel to the middle 2-second segment, significant differences were measured in AdSD patients and normal controls for all parameters, except for the average F0 (all comparisons) and PNR (pretreatment and posttreatment; Table 6). However, in considering all acoustic parameters in the pretreatment-posttreatment comparison, as well as the pretreatment-control and posttreatment-control comparisons, the p values for all medial segments were very similar to the p values for the initial segment (Table 5). The results for the initial and medial segments will therefore not be given separately.

All parameters except the average F0, PNR, and intensity improved significantly after Botox treatment. Before treatment, only the average F0 and intensity did not differ significantly from normal control values. After treatment, the PNR SD and intensity SD could be added to this list. Therefore, the majority of acoustic parameters measured in this study

TABLE 5. TEMPORAL AND ACOUSTIC CHARACTERISTICS IN PATIENTS BEFORE AND AFTER BOTOX TREATMENT AND CONTROLS

		Pretreatment		*Posttreatment*		*Controls*				
		Mean	*IQR*	*Mean*	*IQR*	*Mean*	*IQR*	*p**	*p†*	*p§*
TST AA (s)		3.25	1.43	2.45	0.65	2.06	0.49	<.001	<.001	<.001
TIIT AA (s)		0.75	0.60	0.18	0.37	0.24	0.24	<.001	<.001	.14
TAT AA (s)		2.56	0.90	2.12	0.59	1.74	0.31	<.001	<.001	<.001
VOT (vowel; s)		0.099	0.169	0.097	0.112	0.033	0.029	.30	<.001	<.001
VOT (word; s)		0.062	0.112	0.069	0.048	0.034	0.023	.60	<.001	<.001
MPT (s)		10.2	11.4	9.3	7.7	15.1	9.7	.038	.029	<.001
Voice breaks (proportion)		4.65	10.3	1.05	3.8	0.31	1.2	.001	<.001	.002
Aperiodicity (proportion)		33.7	70.0	4.5	15	1.0	3.8	<.001	<.001	<.001
F0 average (Hz)	i	169.7	43.3	176.0	61.4	171.9	64.3	.49	.79	.75
	m	166.7	46.8	174.6	62.4	175.7	63.7	.67	.96	.95
F0 SD (Hz)	i	9.6	10.4	5.5	5.6	2.8	2.0	.001	<.001	<.001
	m	6.6	6.9	2.0	2.1	1.4	0.9	<.001	<.001	.003
MAS (Hz/s)	i	162.5	138.7	94.4	79.3	52.1	20.8	<.001	<.001	<.001
	m	132.4	122.4	44.2	44.3	42.0	22.8	<.001	<.001	.097
PNR average (dB)	i	11.3	10.2	13.3	7.3	16.6	4.4	.26	<.001	.001
	m	11.1	12.6	14.0	7.4	15.8	5.3	.13	.052	.59
PNR SD (dB)	i	4.2	2.0	3.8	1.5	3.1	1.4	.18	.001	.059
	m	3.2	1.9	2.0	1.6	2.2	2.1	<.001	.003	.32
Intensity average (dB)	i	71.1	8.0	72.1	7.1	73.3	5.2	.99	.36	.53
	m	70.1	8.4	72.5	8.2	68.4	7.2	.77	.26	.092
Intensity SD (dB)	i	3.0	2.0	2.4	1.7	2.1	1.3	.029	.001	.17
	m	1.8	1.8	0.9	0.6	0.9	0.6	.002	.001	.21
Intensity range (dB)	i	15.8	11.6	13.3	10.0	10.8	5.5	.30	<.001	.006
	m	9.1	8.9	4.0	2.8	4.0	2.4	<.001	<.001	.68

i — Initial 2-second segment; m — medial 2-second segment. For explanation of other abbreviations, see text.

*Wilcoxon matched-pairs signed ranks test comparing pretreatment and posttreatment values.

†Mann-Whitney test comparing pretreatment values of patients with values of controls.

§Mann-Whitney test comparing posttreatment values of patients with values of controls.

TABLE 6. VOWEL CHARACTERISTICS OF INITIAL TWO-SECOND SEGMENT VERSUS MEDIAL TWO-SECOND SEGMENT

	Pretreatment	*Posttreatment*	*Controls*
F0 average	.427	.085	.856
F0 SD	.002	<.0001	<.0001
MAS	<.0001	<.0001	.002
PNR average	.161	.056	.019
PNR SD	.001	<.0001	<.0001
Intensity average	.002	<.0001	<.0001
Intensity SD	<.0001	<.0001	<.0001
Intensity range	<.0001	<.0001	<.0001

Data are p values of Wilcoxon matched-pairs signed ranks test. For explanation of abbreviations, see text.

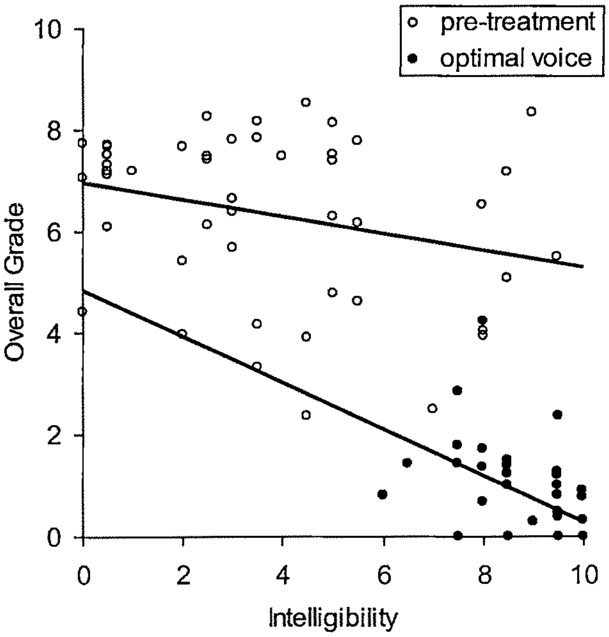
remained significantly different from those of normal controls.

Last, possible relationships among the 3 modalities were assessed, but no strong correlations were detected among the perceptual ratings, acoustic parameters, and self-assessment scores. The highest correlations were found between the pretreatment and posttreatment scores of overall perceptual grade and the patient's self-assessment intelligibility in conversational speech, as is depicted in the Figure (pretreatment, r = –.36, p = .014; posttreatment, r = –.51, p < .001; Spearman's rank correlation coefficient test for nonparametric data).

DISCUSSION

In this prospective study, the actual efficacy of Botox injections in AdSD was assessed by comparing patient's pretreatment values to posttreatment values and comparing both pretreatment and posttreatment values with those of normal controls. Perceptual ratings, (temporal) acoustic analysis, and self-assessment scores were used to achieve this. The posttreatment condition (end point) was defined as the moment the patient experienced his or her voice as normal or as optimal after at least 3 consecutive injections.

The methodological design of this study significantly differs from those of previous studies of AdSD and Botox treatment. Most studies focus on the duration of benefit and side effects, on comparisons of injection techniques (unilateral versus bilateral, peroral versus percutaneous injections), and/or on searching for the lowest efficacious dose. Efficacy is generally evaluated either by comparing patient's pretreatment and posttreatment conditions or by comparing the posttreatment situation with that of normal controls. Moreover, objective tests (often 1 modality) are applied at arbitrary intervals, varying from 1 to 6 weeks, after Botox treatment. In this study, the efficacy of Botox treatment was assessed by concentrating on the optimal voice quality. Therefore, it is hard to compare our results with those available in the literature.



Correlations between overall perceptual grade and patients' self-assessment of intelligibility before and after Botox treatment.

PERCEPTUAL ANALYSIS

Until now, no systematic perceptual rating of the pretreatment *and* posttreatment AdSD voices has been reported. Most studies dealing with evaluation of Botox treatment only use the overall voice quality or rate some features of the "typical" AdSD voice. These include strain, fluency, effort of speaking, and spasm severity, as well as the well-known side effects of Botox treatment such as breathiness and hoarseness. In the present study, the perceptual ratings showed that after treatment, the voice characteristics were significantly improved. However, on average, the posttreatment voices were not perceived as normal. Apparently, perceptually, the characteristic and severely impaired AdSD voice did improve, but another type of pathological voice was detected after Botox treatment.

SUBJECTIVE VOICE EVALUATION

Probably the most important evaluation procedure for determining the success of treatment is the patient's judgment of his or her voice quality and performance. This was already pointed out by Woodson et al: "The patient's perception of his/her problem is, of course, the 'bottom line' in determining patient satisfaction with treatment. These ratings are essential to validate the relevance of objective measures."[13(p342)] In our study, the moment the patient perceived his or her voice as normal or optimal determined the end of the trial. In several other studies, patients' diaries were used to assess the efficacy of Botox treatment and to determine the duration of benefit and side effects. However, available reports on the magnitude of improvement of the investigated subjective parameters are limited.[16,17] Therefore, we know that AdSD patients, as a group, have benefited from Botox treatment, but not to what extent. For example, what does a success rate of 97%, as reported by Inagi et al,[18] really mean — relief of symptoms or achievement of a normal voice? In the current study, patients rated a significant improvement after treatment, but some hoarseness and difficulty with loudness level persisted. Thus, from the patient's perspective, voice quality and performance improved, but were never judged as normal. This outcome is in line with the perceptual ratings: a much better, but still abnormal, voice is being perceived after Botox treatment.

ACOUSTIC ANALYSIS

For reliable assessment of voice quality, one needs reproducible measurements and parameters that can objectively monitor changes in voice quality after treatment. These measurements should, preferably, be relevant to the clinical symptoms. In the past decade, several publications have dealt with AdSD and acoustic analysis. Woodson et al summarize the value of acoustic analysis in this way: "Since acoustic changes are insufficiently specific to separate spasmodic dysphonia from other neurological voice disorders, they should not be regarded as diagnostic tests, but as indicators of function. In patients with spasmodic dysphonia, acoustic analysis is useful in documenting severity and monitoring response to treatment."[13(p340)]

Acoustic evidence of temporal disorganization of AdSD speech has already been reported. In 1997, Cannito et al[7] found that although dysfluency was not a defining feature of spasmodic dysphonia, it did contribute significantly to the overall impression of the severity of the disorder. In a noninterventional study, they found significant differences between normal control subjects and AdSD subjects for the TST, TIIT, and TAT. This is in line with our findings: before treatment, all 3 parameters for the patients significantly differed from those of the normal controls. However, although these parameters improved after treatment, they still remained significantly different from those of the normal controls. Until now, only Ludlow et al[19] and Ford et al[20] had demonstrated that the "mean sentence length" and "time required to read" decreased after Botox intervention. Unfortunately, in both studies, the TIITs were not assessed. Therefore, an increase in sentence duration could also have been the result of a longer pause time between the words.

The following acoustic parameters are routinely used to quantify changes in voice quality: F0, F0 SD, perturbation parameters (jitter, shimmer), harmonics-to-noise ratio, and measurements of voice breaks ("voice break factor," "voice break index," "degree of voice breaks").[21] It was found that these parameters in AdSD patients differed significantly from those of normal controls. After Botox treatment, however, a statistically significant reduction was found only for F0 SD and measurements of voice breaks.[22] Jitter and shimmer were not assessed in our study, because Bielamowicz et al[23] found that these measures could not be reliably applied to voices that were even mildly aperiodic — much less to pathological voices. Similarly, Titze and Liang[24] found that for frequency variations above 6% per cycle, no statements about accuracy could be made.

In 1997, Zwirner et al[8] found significant differences between the initial and middle segments in normal control subjects for the F0 SD. Before treatment, no significant differences in the AdSD group could be detected. The difference between the initial second and the middle second was statistically significant. They therefore concluded that a "normal" pattern of laryngeal stability was achieved during sustained phonation. In the current study, the initial 2 seconds differed significantly from the medial 2-second segment extracted from the sustained vowel for all acoustic parameters (including F0 SD) for both the pretreatment and posttreatment conditions, except for the average F0 and PNR (pretreatment; Table 6). That our results already differed significantly in the pretreatment condition is in contrast to the results of Zwirner et al. However, only 16 subjects participated in their study. Moreover, it is unclear which pitch extraction algorithm was used, or whether the extracted segments were provided with linear ramped offsets to avoid abrupt audible signal transition.

In this study, (temporal) acoustic analysis revealed that there was a significant improvement after Botox treatment. Nevertheless, the "optimally" treated AdSD voice still remained deviant as compared to normal voice quality. Acoustically, the typical AdSD characteristics persisted, although they were present to a lesser extent.

RELATIONSHIP BETWEEN MODALITIES

Although acoustic analysis of the voice can provide objective and reproducible measures of phonation, there are no specific parameters that can acoustically characterize AdSD. The clinical relevance of these objective measures could be demonstrated if a strong association existed with the more subjective measurements of vocal function and performance made by the patient and the observer/listener. In this study, however, no strong correlations were found among the perceptual ratings, acoustic parameters, and self-assessment scores. Zwirner et al[12] could not detect specific relationships among any of the acoustic parameters and/or the airflow rate and/or the videoendoscopic findings.

CONCLUSION

Currently, Botox injection is the therapy of first choice for AdSD. Although significant improvement is measured perceptually, subjectively, and acoustically, in general, the optimal voice obtained with Botox is never fully normal in quality or function.

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BRONCHIAL INTRAEPITHELIAL NEOPLASTIC LESIONS IN HEAD AND NECK CANCER PATIENTS: RESULTS OF AUTOFLUORESCENCE BRONCHOSCOPY

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To determine the efficacy of autofluorescence bronchoscopy for detection of bronchial intraepithelial neoplastic lesions in head and neck cancer patients, we analyzed data from head and neck cancer patients who underwent both white light bronchoscopy and autofluorescence bronchoscopy for the rates of detection of intraepithelial neoplastic lesions. The results of the histopathologic examination were compared with the bronchoscopic findings. The sensitivity for detection of intraepithelial neoplastic lesions was calculated. Eleven moderate dysplasias and 3 severe dysplasias were detected during 8 of the 42 bronchoscopic examinations (19%) in 6 of the 24 patients (25%). The sensitivities for white light bronchoscopy alone and for white light bronchoscopy combined with autofluorescence bronchoscopy for detection of intraepithelial neoplastic lesions were, respectively, 21% (3 of 14) and 57% (8 of 14). In short, bronchial intraepithelial neoplastic lesions were found in a considerable percentage of head and neck cancer patients. Use of autofluorescence bronchoscopy improved the detection of these lesions.

KEY WORDS — autofluorescence, bronchoscopy, early detection, head and neck neoplasms, lung neoplasms, multiple primary neoplasms.

INTRODUCTION

Patients with head and neck squamous cell carcinoma (HNSCC) are at risk for second malignant tumors, and many of these second tumors arise in the lungs.[1-6] This finding supports the field cancerization theory, which states that multiple cell groups independently undergo neoplastic transformation under the stress of regional carcinogenic activity.[7] The probability of the development of lung cancer within 15 years from the diagnosis of HNSCC is reported to be as high as 10% to 37%, depending on the site of the index tumor, with the highest risk for patients with laryngeal carcinoma.[1] Most of these second primary lung tumors are squamous cell carcinomas.[2,6,8] Preneoplastic lesions are rarely found in bronchial biopsy specimens of patients with a history of laryngeal carcinoma.[9] However, bronchial intraepithelial neoplastic lesions are difficult to detect during conventional white light bronchoscopy. The reported sensitivity for detection of these lesions varies between 9% and 59%.[10-16] Studies on autofluorescence bronchoscopy have consistently shown that it can improve the detection of intraepithelial neoplastic lesions compared with white light bronchoscopy in the population at risk for bronchial neoplasia.[10-18] Therefore, more premalignant changes of the airway epithelium may be found when autofluorescence bronchoscopy is used in HNSCC patients.[19] We analyzed the data of HNSCC patients who underwent autofluorescence bronchoscopy. The data were examined for the detection and course of intraepithelial neoplastic lesions. The detection of these lesions is considered important because they may progress to invasive cancer.[20-22]

MATERIAL AND METHODS

The study population consisted of all patients from the Department of Pulmonary Medicine of the University Hospital Vrije Universiteit in Amsterdam with a history of HNSCC who underwent both white light bronchoscopy and autofluorescence bronchoscopy. Some of the patients participated in a multicenter study investigating the prevalence of bronchial dysplasia and carcinoma in situ using autofluorescence bronchoscopy and conventional bronchoscopy. The indications for autofluorescence bronchoscopy were suspected cancer, staging of newly diagnosed lung cancer or follow-up of lung cancer, or HNSCC. The patients were included in this analysis only when bronchial biopsies were performed and when a matching pair of white light and autofluorescence assessments was available.

All bronchoscopic examinations were performed by the same bronchoscopist (T.G.S.). The methods

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PATIENT DEMOGRAPHICS, CLINICAL DATA, AND BRONCHOSCOPY DATA

No. of patients	24
M/F	23/1
Site of HNSCC (No. of patients)	
Oral cavity	4 (17%)
Oropharynx	2 (8%)
Hypopharynx	4 (17%)
Larynx	13 (54%)
Unknown origin	1 (4%)
Time from HNSCC to first bronchoscopy (No. of patients)	
<6 mo	3 (12%)
6 mo to 2 y	5 (21%)
>2 y	16 (67%)
Age at bronchoscopy (median and range)	65.5 (43-81)
Total No. of bronchoscopies	42
No. of bronchoscopies per patient (median and range)	1 (1-6)
Indications for first bronchoscopy (No. of patients)	
Follow-up of HNSCC	2 (8%)
Follow-up of HNSCC and lung cancer	2 (8%)
Staging of lung cancer	1 (4%)
Pulmonary lesion(s) on chest X-ray	14 (59%)
Hemoptysis	5 (21%)
Total No. of biopsies	199
No. of biopsies per bronchoscopy (median and range)	4.5 (2-11)

HNSCC — head and neck squamous cell carcinoma.

of the bronchoscopic examination and of the processing and histologic classification of the bronchial biopsy specimens have been described previously.[16,18]

The data were examined for the detection and course of intraepithelial neoplastic lesions. Intraepithelial neoplastic lesions were defined as lesions that were scored by the pathologist as moderate dysplasia, severe dysplasia, or carcinoma in situ. Analysis was performed on a per-patient basis and on a per-bronchoscopy basis. The results of the histopathologic examination were compared with the results of the visual assessment using the white light and the autofluorescence cameras. The sensitivities for detection of the total number of moderate dysplasias, severe dysplasias, and carcinomas in situ for white light bronchoscopy alone and for white light bronchoscopy combined with autofluorescence bronchoscopy were calculated.

RESULTS

From November 1995 to December 1998, a total of 42 white light bronchoscopic and autofluorescence bronchoscopic examinations were performed in 24 patients who fulfilled the inclusion criteria. Patient demographics, clinical data, and bronchoscopy data are shown in the Table. Of the total number of biopsy specimens obtained, 90% (179 of 199) were judged by the pathologists to be satisfactory, enabling them to examine the morphology adequately.

A total of 14 intraepithelial neoplastic lesions were detected during 8 of the 42 bronchoscopic examinations (19%). These lesions were found in 6 of the 24 patients (25%), and consisted of 11 moderate dysplasias and 3 severe dysplasias. One severe dysplasia was seen only during white light bronchoscopy and was missed during autofluorescence bronchoscopy. Five moderate dysplasias were seen only during autofluorescence bronchoscopy. One severe dysplasia and 5 moderate dysplasias were missed by both methods. These sites were sampled for biopsy because we always take a biopsy specimen from an area assessed as normal during both white light bronchoscopy and autofluorescence bronchoscopy.[16,18] Results from follow-up biopsy specimens were available for 6 of the 14 sites showing intraepithelial neoplastic changes. Four sites with moderate dysplasia showed mild dysplasia in follow-up biopsy specimens. Two sites with severe dysplasia both showed mild dysplasia in later biopsy specimens. One of these severe dysplastic sites showed squamous metaplasia and the other showed normal epithelium in the last biopsy specimens taken.

The sensitivities of white light bronchoscopy alone and of white light bronchoscopy combined with autofluorescence bronchoscopy for detection of intraepithelial neoplastic lesions were, respectively, 21% (3 of 14) and 57% (8 of 14).

DISCUSSION

It is important to note that this study was performed in a selected patient population, because most patients were suspected of having a second primary lung cancer. A total of 14 intraepithelial neoplastic lesions was found in 25% of patients with a history of HNSCC, including 11 moderate dysplasias and 3 severe dysplasias. The detection of these lesions is considered important because previous studies showed that approximately 10% of moderate dysplasias and 19% to 46% of severe dysplasias will progress to invasive cancer.[20-22] However, all of the neoplastic sites from which follow-up bronchial biopsy specimens were available showed a milder degree of dysplasia or no dysplasia at all during follow-up.

There may be several reasons for this finding. The whole lesion may have been mechanically removed by a previous biopsy procedure. Spontaneous regression of neoplastic changes may also have occurred. This has been observed before in clinical and experimental settings.[23-25] In a short-term follow-up study reported by Thiberville et al,[23] regression of 15 of 24 severe dysplasias (63%) and of 7 of 32 carcinomas in situ (22%) was documented at 3 months after diagnosis. One severe dysplasia and 7 carcinomas in situ progressed. Observer variability in the histopathologic reporting of bronchial biopsy specimens may also be an explanation.[26,27] Another reason may be sampling error.

The precise outcome of the lesions remains unknown. Follow-up is necessary to find out whether some of these lesions will progress to severe neoplasia. When progressive neoplastic changes occur, the lesions can be treated with curative intent by use of currently available methods for endobronchial treatment such as photodynamic therapy or electrocautery.[28-33]

The sensitivities for detection of intraepithelial neoplastic lesions were 21% for white light bronchoscopy alone and 57% for white light bronchoscopy combined with autofluorescence bronchoscopy. In a previous study, we reported sensitivities for white light bronchoscopy alone and for white light bronchoscopy combined with autofluorescence bronchoscopy of, respectively, 59% and 85%.[16] The most likely explanation for the lower sensitivity in the present study is the fact that 11 of the 14 intraepithelial neoplastic lesions were moderate dysplasias. Moderate dysplasia is the intraepithelial neoplastic lesion most frequently missed by both white light bronchoscopy and autofluorescence bronchoscopy.[16]

We conclude that bronchial intraepithelial neoplastic lesions were found in a considerable number of patients with a history of HNSCC. Use of autofluorescence bronchoscopy improved the detection of these lesions.

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AIRFLOW RESISTANCE OF AIRFLOW-REGULATING DEVICES DESCRIBED BY INDEPENDENT COEFFICIENTS

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Rehabilitation after laryngectomy includes more and more the use of airflow-regulating devices such as shunt valves (SVs), tracheostoma valves (TSVs), and heat and moisture exchange (HME) filters. In determining the quality of those devices, airflow resistance is a very important factor. It is currently defined as pressure drop divided by airflow. However, for most applications, this definition does not result in a pressure- and airflow-independent parameter. Therefore, a new set of parameters is defined and applied to pressure-airflow curves of airflow-regulating devices. Pressure drop over TSVs and HME filters appears to have a squared relationship with flow. In SVs, it has a linear relationship. The new set of parameters describes the pressure-airflow relationship properly for all considered devices. In conclusion, theoretical predictions of flow mechanics appear to be valid for SVs, TSVs, and HME filters. Only 2 coefficients are necessary to describe the pressure-flow characteristics of these airflow-regulating devices, independent of pressure drop over and flow through the device.

KEY WORDS — airflow resistance, heat and moisture exchange filter, laryngectomy, pressure drop, shunt valve, tracheostoma valve.

INTRODUCTION

The treatment of a malignant tumor in the laryngeal area often consists of surgical removal of the larynx (laryngectomy), including the vocal folds and epiglottis. Reconstruction results in separation of the airway and digestive tract (Fig 1). The trachea is led outside and ends in a tracheostoma. During the operation, a fistula is created between the trachea and esophagus. In the fistula, a shunt valve is placed.[1] The shunt valve prevents food and fluid from entering the trachea and allows airflow from the trachea to the esophagus. Speech is made possible by closing the tracheostoma manually; expired air then flows via the shunt valve through the upper esophageal sphincter, which starts to vibrate and serves as a substitute for the vocal folds (pseudoglottis). Several types of shunt valve are available on the market, such as the Provox[2] and the Groningen button,[3] which are used regularly in spite of their limited lifetime.[4,5]

When the skin around the tracheostoma is sufficiently flat, a tracheostoma valve can be used.[6-11] It is glued onto the skin around the tracheostoma. Hands-free speech is made possible by producing a spurt of air that closes the tracheostoma valve. To improve the rehabilitation process, a heat and moisture exchange (HME) filter can be applied. It moisturizes and warms the inhaled air[12,13] to prevent the tracheal tissues from drying out, and thus reduces excessive mucus production and the occurrence of crusts in the trachea. Also, by raising airflow resistance, the HME filter increases the tissue oxygen saturation.[14] An HME filter can be attached to a tracheostoma valve or applied independently by gluing it onto the skin like a tracheostoma valve.

An aspect common to all the devices mentioned above is airflow regulation. During airflow passage, they regulate the humidity and temperature (HME filters) or regulate the direction of the airflow (tracheostoma valve, shunt valve). One of the factors that determines the quality of these airflow-regulating

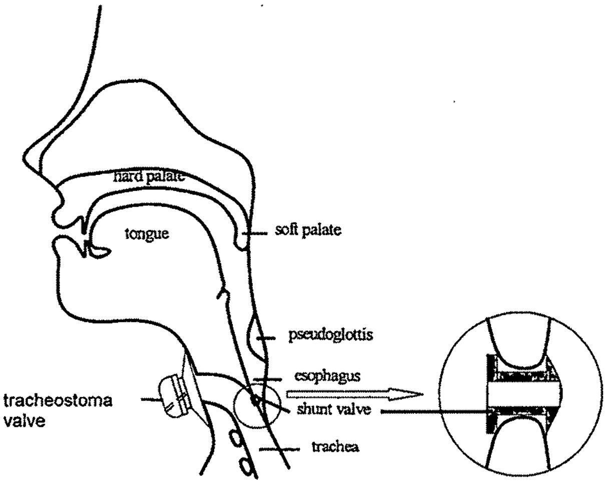

Fig 1. Schematic drawing of rehabilitation process of laryngectomized patients using shunt valve and tracheostoma valve.

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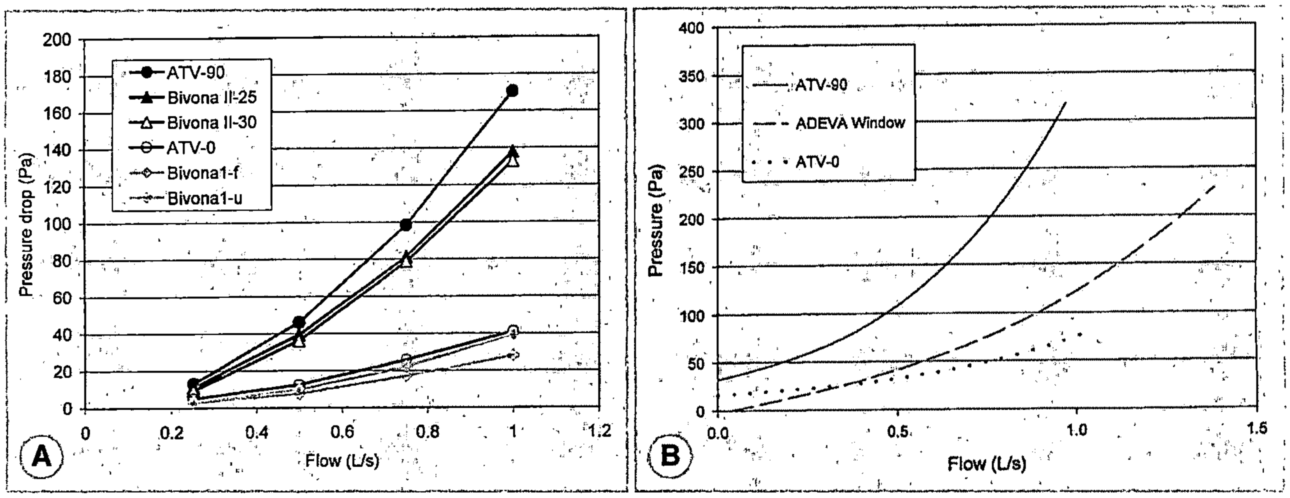

Fig 2. Relationship between pressure drop over and flow through tracheostoma valves, as reported by **A)** Grolman et al[22] and **B)** Verkerke et al[23] (third-order regression).

devices is the pressure drop over the device. Devices with a high pressure drop require a high pressure to produce a certain airflow; thus, much energy is needed for the patient to inhale and exhale. When realization of these high pressures is not possible, a limited airflow will result, which might cause problems in breathing. Pressure drop depends strongly on airflow, so a more independent parameter, airflow resistance, is used to quantitate it.[5,15-18]

Airflow resistance is often defined as the pressure drop over the device divided by the airflow through the device, analogous to the electrical resistance of an electrical device, which is defined as the voltage drop over the device divided by the direct current through the device. Electrical resistance defined in this way appears to be a constant. That is, an increased voltage results in an increased current such that the quotient of voltage and current remains unchanged; thus, electrical resistance is independent of voltage and current. Electrical resistance is therefore an appropriate parameter for describing electrical behavior.

However, airflow resistance is a more complex phenomenon. Obtaining an independent parameter for airflow resistance by simply dividing pressure drop by airflow does not automatically result in a constant that is valid for all possible airflow and pressure values. A correct interpretation of a given flow resistance value is only possible in combination with flow values, but often, this combination is lacking, as in the articles of Cole et al,[19] McRae et al,[20] and Smitheran and Hixon.[21]

The aim of this article is to provide more insight into airflow behavior and description and to define a set of parameters that is appropriate for describing the airflow resistance of airflow-regulating devices for patients who have undergone laryngectomy. For each type of device (tracheostoma valves, HME filters, and shunt valves), the most appropriate parameter was derived and checked for its independence by use of existing information about pressure-flow relationships.

MATERIALS AND METHODS

Recently, extensive studies on the airflow and pressure characteristics of airflow-regulating devices have been performed.[22-25] Four tracheostoma valves have been analyzed: the Blom-Singer ATV (Adjustable Tracheostoma Valve, Inhealth Technologies, Carpinteria, California); the Bivona I and the Bivona II tracheostoma valves (Bivona Medical Technologies, Gary, Indiana); and the Adeva Window (Adeva Medical, Lübeck, Germany). Four HME filters have been analyzed: the Blom-Singer HumidiFilter (Inhealth Technologies); the Provox stoma filter (Atos Medical AB, Hörby, Sweden); the Free Vent (Pharma Systems AB, Knivsta, Sweden); and the Stom-Vent 2 (Louis Gibeck AB, Upplands Väsby, Sweden). Five shunt valves have been analyzed: the Blom-Singer indwelling low-pressure voice prosthesis (Gelcap, Inhealth Technologies); the Groningen Ultra Low Resistance (Medin Instruments, Groningen, the Netherlands); the Provox and the Provox-2 (Atos Medical AB); and the VoiceMaster (Entermed, Woerden, the Netherlands).

Figures 2-4[22-25] show the relationships between the pressure loss and flow of the analyzed tracheostoma valves, HME filters, and shunt valves, respectively. Data derived from these graphs will be used in this discussion.

For each type of device, a numerical description of the relationship between airflow through the device and pressure drop over the device will be made.

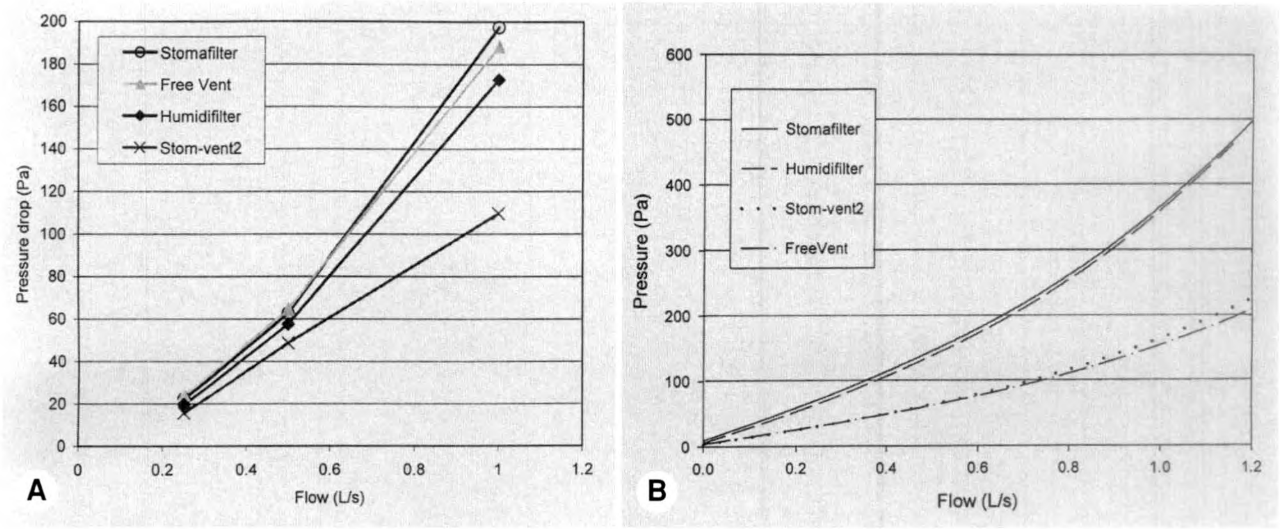

Fig 3. Relationship between pressure drop over and flow through heat and moisture exchange filters, as reported by **A**) Grolman et al[24] and **B**) Verkerke et al[23] (third-order regression).

The numerical description will be used to define a parameter that is independent of airflow and pressure drop. For each device, the value of this parameter will be calculated from the data in Figs 2-4. A graph of the parameter values plotted against the airflow will demonstrate whether the parameter is indeed independent of airflow and pressure drop. In general, the simplest way to describe the relationship between pressure drop and airflow is given by the modified Bernoulli equation[26]:

(1) $\Delta P = {}^{1}/_{2} \cdot \rho \cdot v^2 \cdot (\lambda \cdot L/d + K)$

with

- ΔP = pressure drop over the considered region [Pa]
- ρ = air density [kg/m^3]
- v = mean airflow velocity = $4 \cdot Q/\pi \cdot d^2$ [m/s]
- Q = airflow [m^3/s]
- d = internal diameter of the considered region [m]
- λ = airflow resistance factor = $64/Re$ [–], when the airflow is linear ($Re < 2{,}300$); = $0.316/Re^{0.25}$ [–], when the airflow is turbulent ($Re > 2{,}300$)
- Re = Reynolds number = $\rho \cdot v \cdot d/\eta$ [–]
- η = dynamic airflow viscosity [Pa • s]
- L = length of the considered region [m]
- K = airflow resistance factor, caused by a change in diameter from D to d ($D > d$) = $(1 - d^2/D^2)^2$ [–]

Some assumptions are included in this equation:

1. Airflow viscosity is constant (= $17.1 \cdot 10^{-6}$ Pa • s).
2. Air density is constant (= 1.29 kg/m^3).
3. Pressure is constant in a plane perpendicular to the streamline.

For shunt valves, we have to consider an additional pressure drop caused by the elastic deformation of the valve. The pressure that is necessary to deform the valve in order to open it can be described by Hooke's law.[27] This implies a linear relationship between pressure drop and airflow: $\Delta P = c \cdot Q$. For shunt valves, formula 1 becomes:

(2) $\Delta P = {}^{1}/_{2} \cdot \rho \cdot v^2 \cdot (\lambda \cdot L/d + K) + c \cdot Q$

with c = elastic constant, depending on valve size and material [Pa • s/m^3]. When all constant factors are integrated, formula 2 becomes

(3) $\Delta P = ARC1 \cdot Q + ARC2 \cdot Q^2 + ARC3 \cdot Q^{1.75}$

with

- ARC1 = airflow resistance coefficient caused by wall friction of a laminar airflow or by deformation of a valve
- ARC2 = airflow resistance coefficient caused by a

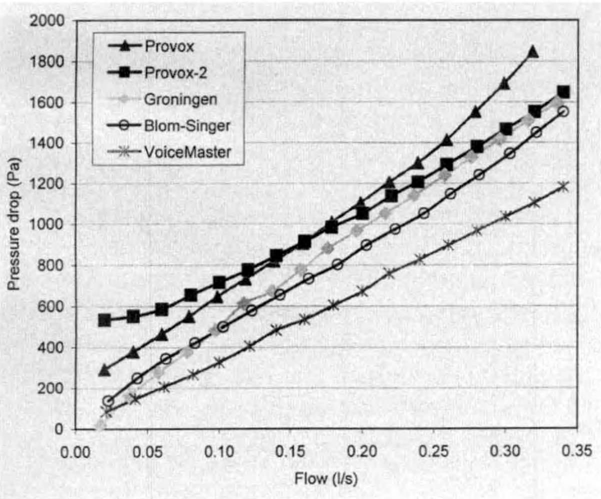

Fig 4. Relationship between pressure drop over and flow through shunt valves, as reported by Grolman.[25]

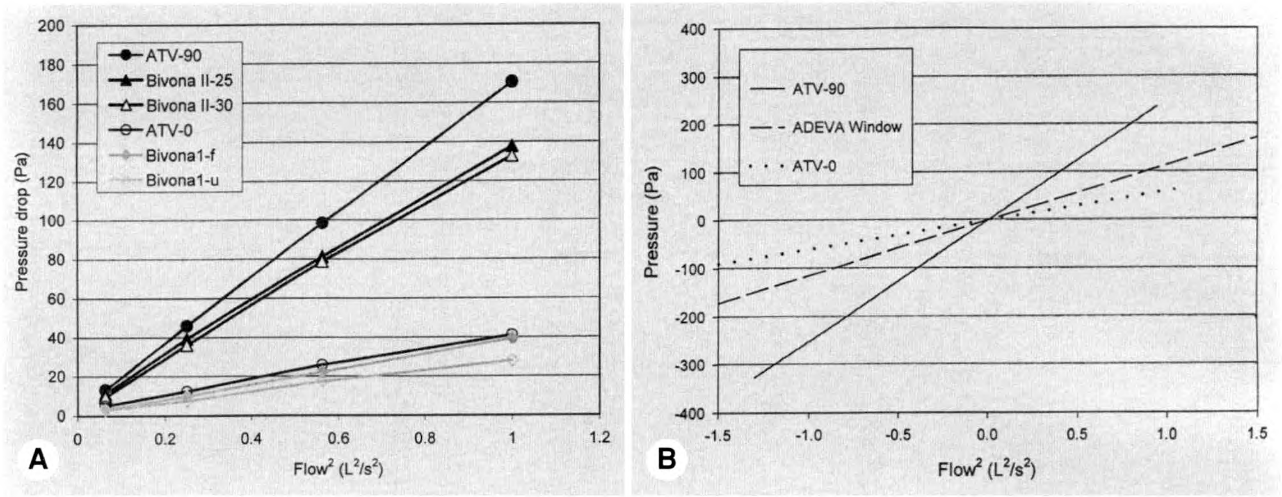

Fig 5. Relationship between pressure drop over and squared flow through 2 most extreme positions of tracheostoma valves, derived from data of **A**) Grolman et al[24] and **B**) Verkerke et al.[23]

change in diameter

- ARC3 = turbulent airflow resistance coefficient caused by wall friction

These 3 potential airflow resistance parameters will be used to describe the airflow behavior of the considered devices.

RESULTS

For tracheostoma valves, formula 2 can only be dominated by wall friction or by friction caused by a change in diameter (the trachea has an internal diameter of about 25 mm, and the internal lumen of the tracheostoma valve has a smaller diameter), since there is no active valve present (c = 0). When the internal lumen of a tracheostoma valve is assumed to be circular with a diameter of 10 mm and a shaft length of 20 mm, then according to formula 2, the pressure drop of a change in diameter is 73.9 Pa, which is more than 10 times higher than the pressure drop caused by wall friction (6.7 Pa). The resulting total pressure drop (80.5 Pa at a flow of 1 L/s) resembles that of Fig 2, so the numerical calculation seems to be correct. Therefore, airflow resistance is best described by ARC2. In Fig 5,[23,24] pressure drop is plotted against the squared airflow values by use of data in Fig 2. Good linear relationships, and thus, rather constant ARC2 values, result. Table 1 presents the ARC2 values of the considered tracheostoma valves for these airflows.

TABLE 1. ARC2 OF TRACHEOSTOMA VALVES FOR FOUR VALVE POSITIONS DERIVED FROM DATA IN FIGURE 2

Tracheostoma Valve and Position	*ARC2*
Bivona I-f	39
Bivona I-m	35
Bivona I-l	38
Bivona I-u	29
ATV-0	43
ATV-0	*60*
ATV-30	67
ATV-60	122
ATV-90	172
ATV-90	*260*
Bivona II-30	139
Bivona II-25	146
Bivona II-20	140
Bivona II-15	136
Adeva Window	*110*

ARC2 is in Pa • s^2/L^2. Data from Fig 2B are in italics. Valve positions are indicated by f, m, l, u for Bivona I; 0, 30, 60, 90 for ATV; and 30, 25, 20, 15 for Bivona II.

The airflow resistance of HME filters is likewise dominated by wall friction or by friction caused by a change in diameter. Wall friction is caused by an airflow that comes into contact with the walls of the little canals in the foam. A change in diameter is caused by the fact that the total cross-sectional area of an HME filter through which air can flow (the summation of all the little canals) is much smaller than the cross-sectional area of the trachea. To analyze which cause is dominant, the flow behavior of the foam is modeled by considering a composition of numerous little canals placed parallel to each other. The length of a canal is considered to be twice the foam thickness, simulating its capricious orientation. By adding the internal surfaces of all canals, the diameter of an imaginary lumen with the same surface can be calculated. This lumen diameter is used to estimate the dominant cause of friction.

When assuming an internal canal diameter of 1 mm, the pressure drop calculated with formula 2 is 132.4 Pa, which resembles the pressures of Fig 3, thus validating the model. It appears that the pressure

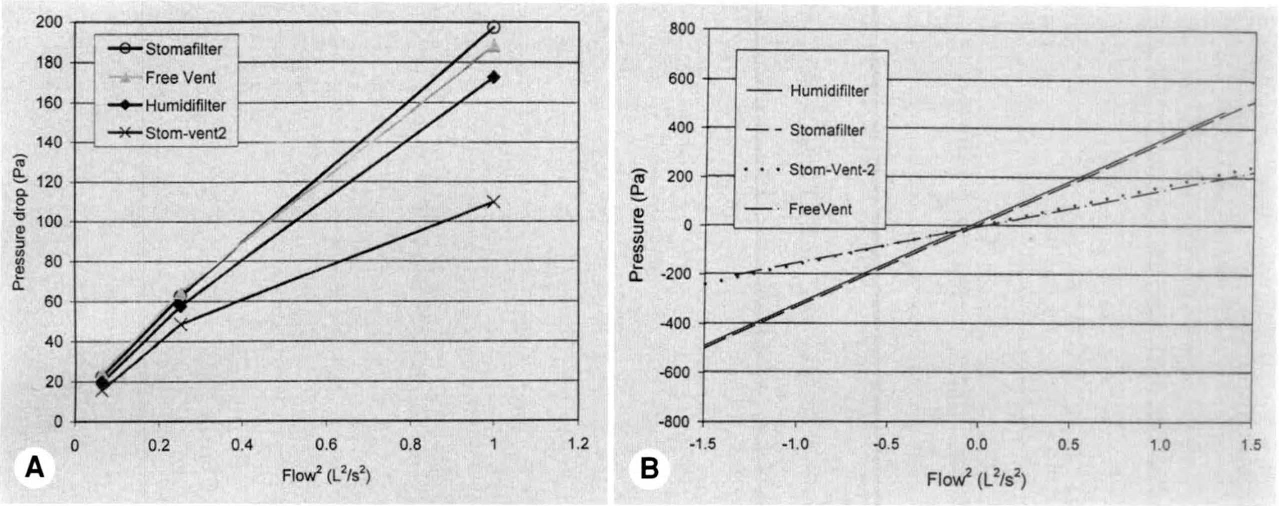

Fig 6. Relationship between pressure drop over and squared flow through heat and moisture exchange filters, derived from data of **A**) Grolman et al[24] and **B**) Verkerke et al.[23]

drop caused by a change in diameter (from tracheal size to the imaginary lumen) is 131.3 Pa, which is more than 100 times higher than the pressure drop caused by friction (1.1 Pa), so again, airflow resistance is best described by ARC2. In Fig 6,[23,24] pressure drop is plotted against squared airflow by use of the data in Fig 3. When the data of Grolman et al[24] are used, no linear relationship appears. However, using the data of Verkerke et al[23] results in the expected linear relationship. The ARC2 values that are presented in Table 2 are based on the data of Verkerke et al.[23]

In shunt valves, formula 2 can be dominated by all 3 elements: wall friction, friction caused by a change in diameter (the trachea has an internal diameter of about 25 mm, and the internal lumen of the shunt valve is narrower), and elastic deformation of the valve. Most shunt valves can be characterized by an internal diameter of 5 mm and a shaft length of 10 mm. By use of these dimensions, the change in diameter appears responsible for 138.9 Pa, which is 9% of the total pressure drop mentioned in Fig 4, and wall friction is responsible for 10.9 Pa, which is 1% of the total pressure drop, so valve deformation will dominate the total airflow resistance. Airflow resistance is therefore best described by ARC1.

Figure 4 shows that for 3 of the 5 shunt valve types, nearly linear relationships exist. Table 3 presents the ARC1 values. The Provox and Provox-2 show a linear behavior only at certain flow intervals (Fig 7): for the Provox-2, >0.05 L/s, and for the Provox, <0.25 L/s. The ARC1 values of Table 3 are only valid for these flows.

DISCUSSION

Airflow-regulating devices are essential in today's rehabilitation of patients who have undergone laryngectomy. The airflow resistance of these devices is a very important characteristic. The lower the airflow resistance is, the higher the comfort for the patient will be. Before airflow resistance can be decreased by changing the design of the device and in order to compare the different devices or models, airflow resistance must be understood and described by pressure- and flow-independent parameters.

Wheatley et al[28] described the 3 possible relationships between pressure drop and airflow, but only considered their separate appearance, not their simultaneous appearance. Rohrer's equation[29] considers only 2 possible relationships: a linear one and a quadratic one.

The presented formula 2 clearly describes total airflow behavior for all the considered airflow-regulating devices. However, in general, each formula is a simplification of reality. In the formula presented,

TABLE 2. ARC2 OF HME FILTERS DERIVED FROM DATA OF VERKERKE ET AL[23] IN FIGURE 3

HME Filter	*ARC2*
HumidiFilter	340
Provox	340
Free Vent	150
Stom-Vent 2	160

HME — heat and moisture exchanger.
ARC2 is in Pa • s²/L².

TABLE 3. ARC1 OF SHUNT VALVES DERIVED FROM DATA IN FIGURES 4 AND 7

Shunt Valve	*ARC1*
Provox	4,600
Provox-2	3,760
Groningen	4,830
Blom-Singer	4,260
VoiceMaster	3,470

ARC1 is in Pa • s/L.

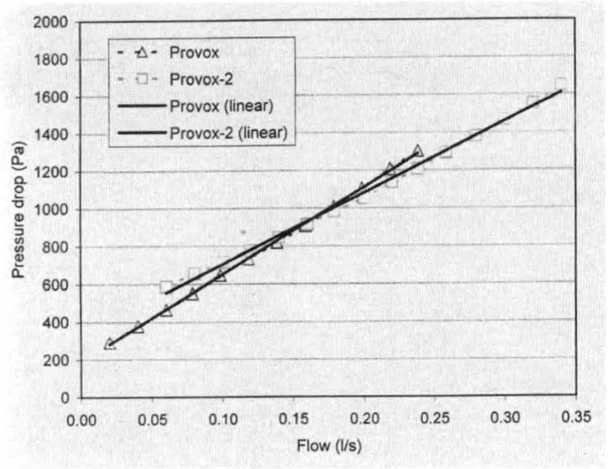

Fig 7. Intervals of linear relationship between pressure drop over and flow through Provox and Provox-2 shunt valves.

airflow viscosity and air density are considered to be constant. Air viscosity depends mainly on temperature, which remains constant within small margins. Air can be considered incompressible for air velocities up to 70 m/s, a limit that is not reached in this study, because the air velocities in the presented devices calculated with formula 1 are 18 m/s at the highest. The assumption that pressure is constant in a plane perpendicular to the streamline is acceptable for the considered geometry.[26]

The data for tracheostoma valves in Fig 5 show that the assumed relationship between airflow and pressure drop exists, especially for flows of 0.5 L/s and higher, so pressure drop is indeed caused by a change in diameter. Wall friction is less important, because the air velocity is relatively low and the length of tracheostoma valves is short. Figure 5 and Table 1 show that a large variation in airflow resistance exists. From formula 2 can be derived that airflow resistance decreases up to 16 times when the lumen diameter is doubled. So, the large variation in airflow resistance is, most probably, caused by small variations in lumen diameter.

When we use the data of Grolman et al,[24] HME filters do not behave as expected. However, the measurements were performed at only 3 different airflows, so it is difficult to draw distinct conclusions. Using the data of Verkerke et al,[23] who included a greater number of different airflows, resulted in the expected constant ARC2 values. When we compare the results in both publications, we find the data of Verkerke et al to be up to 2 times higher. The reason for this difference cannot be explained.

Shunt valves generally show, indeed, the expected behavior, so the assumed dominance of elastic valve deformation in the airflow resistance exists. However, the 2 Provox devices show a somewhat different behavior. First, high opening pressures exist, probably because the valves stick to the housing. Second, the relationship between pressure and flow is not linear, but has a quadratic component. This is most probably because the inner diameter is restricted in comparison to that of other shunt valves, and because the valve has a small hinge that requires less elastic deformation during valve opening. According to formula 1, the contribution to the total friction of wall friction and friction caused by a change in diameter will rise from 10% to 25%, thus explaining the more quadratic behavior. The range in which rather constant ARC2 values exists is from 0.05 to 0.25 L/s, which falls within the physiological range. Since no device is dominated by wall friction, ARC3 is not necessary for describing airflow resistance, and 2 parameters, ARC1 and ARC2, are sufficient.

CONCLUSION

A practical set of 2 coefficients (ARC1 and ARC2) has been defined to describe airflow resistance. The advantage of these coefficients is that they are independent of airflow and pressure. Comparing devices can be performed by comparing the coefficient values instead of comparing pressure-flow diagrams. ARC1 is defined by pressure divided by flow; ARC2 is defined by pressure divided by *squared* flow. It appeared possible to characterize shunt valves (except Provox) by ARC1 and tracheostoma valves and HME filters by ARC2. Because, for each device type, the main cause of airflow resistance has been analyzed, an efficient improvement of existing devices or designing of new devices becomes possible.

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Ann Otol Rhinol Laryngol 110:2001

ORAL ADMINISTRATION OF COLLAGEN CONJUGATED WITH CHOLERA TOXIN INDUCES TOLERANCE TO TYPE II COLLAGEN AND SUPPRESSES CHONDRITIS IN AN ANIMAL MODEL OF AUTOIMMUNE EAR DISEASE

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B10.RIII (H-2^r) mice were orally administered cyanogen bromide peptide 11 (CB11) or cholera toxin B (CTB)–conjugated CB11 to induce tolerance in collagen-induced autoimmune ear disease. Oral administration of a high dosage of CB11 provided partial protection from chondritis. However, administration of a tiny amount of CTB-CB11 conjugate effectively suppressed chondritis. Oral administration of CTB-CB11 conjugate did not alter the stimulation of T cells in vitro or the fine specificities of B cells. The oral administration of CTB-CB11 caused a higher level of type II collagen–specific IgG and its subclass. Interestingly, increases of TH1 cytokine (interferon-γ) in Peyer's patches and of TH1/TH2 cytokines (interleukin-2 and interleukin-4) in lymph nodes were detected in mice that had been fed CTB-CB11. An increase of $CD8^+$ T cells in the Peyer's patches with a decrease of $CD8^+$ T cells in lymph nodes was seen in mice that had been fed CTB-CB11. These results suggest that protection from chondritis by oral administration of minute amounts of CTB-CB11 conjugate can be achieved by a mechanism distinct from that of conventional oral tolerance induction.

KEY WORDS — cholera toxin B subunit, chondritis, oral tolerance, type II collagen.

INTRODUCTION

Oral induction of tolerance has been recognized as an effective method of treating allergy. This method has been successfully applied in autoimmune diseases such as experimental allergic encephalomyelitis,[1-6] collagen-induced arthritis,[7-12] uveitis,[13-15] and type I diabetes mellitus.[16,17] Although oral administration offers a good method for inducing immunologic tolerance, it requires large amounts of antigen or multiple small doses of antigen.

Cholera toxin is an enterotoxin produced by the pathogenic bacteria *Vibrio cholerae*. It has been characterized as an 88 kd protein composed of A and B subunits. Cholera toxin B (CTB) has functions in mucosal binding through the membrane GM1 ganglioside and lacks the ability to elevate cyclic adenosine monophosphate.[18] It has been shown in vitro to modulate T cell–mediated immune responses.[18,19] The application of CTB as a transmucosal antigen delivery system to induce tolerance has been reported in delayed-type hypersensitivity lymphocyte proliferation and antibody responses.[6,20] Oral administration of CTB-conjugated myelin basic protein has been shown to induce tolerance in mice with experimental allergic encephalomyelitis,[6] and CTB-conjugated insulin did the same in nonobese diabetic mice.[17] These studies showed that oral administration of small, rather than large, amounts of CTB-conjugated antigens can successfully suppress autoimmune diseases.

Collagen autoimmunity has been described not only in arthritis, but also in several ear diseases, including auricular chondritis, Meniere's disease, otosclerosis, Cogan's syndrome, and autoimmune sensorineural hearing loss.[21-25] Collagen-induced autoimmune ear diseases in animals show histopathologic and immunologic resemblance to human autoimmune ear diseases.[26-28] Auricular chondritis induced in rats by immunization with type II collagen (CII) was reported as an animal model for relapsing polychondritis in humans.[23] Although both diseases are associated with high levels of humoral and cellular autoimmunity to CII, the role of antibodies and T lymphocytes in the pathogenesis of these diseases is not adequately understood.[25-27] The cyanogen bromide peptide 11 (CB11) of bovine CII carries epitope(s) for inducing auricular chondritis.[29] In the study reported here, we orally administered CB11 or CTB-CB11 conjugate to B10.RIII mice before sensitization with

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bovine CII. This conjugate induced tolerance to collagen in collagen-induced chondritis.

MATERIALS AND METHODS

Bovine Type II Collagen, Cyanogen Bromide Peptides, and Mice. Bovine type II collagen and bovine cyanogen bromide peptides 8, 9, 10, and 11 were prepared from bovine collagen by previously described methods.[30,31] All of the sequences of cyanogen bromide peptides have been established.[30] B10.RIII mice ($H\text{-}2^r$) at the age of 6 to 10 weeks were purchased from Jackson Laboratory (Bar Harbor, Maine) and bred at the University of Tennessee (Memphis, Tennessee).

Culture Media. Serum-free Ventrex HL-1 medium (Ventrex Laboratories, Portland, Maine) was supplemented with 1-mmol/L sodium pyruvate, 100-µg/mL penicillin, 100-µg/mL streptomycin, 2-mmol/L glutamine, 5×10^{-5}-mol/L 2-mercaptoethanol, 20-mmol/L HEPES (pH 7.4), and 1X nonessential amino acids. RPMI medium 1640 was supplemented with 10% fetal calf serum (Hyclone Laboratory, Logan, Utah), 1-mmol/L sodium pyruvate, 100-µg/mL penicillin, 100-µg/mL streptomycin, 2-mmol/L glutamine, 5×10^{-5}-mol/L 2-mercaptoethanol, 20-mmol/L HEPES (pH 7.4), and 1X nonessential amino acids. The RPMI medium 1640, sodium pyruvate, penicillin, streptomycin, glutamine, HEPES, and 50X nonessential amino acids were purchased from Irvine Scientific (Santa Ana, California). The 2-mercaptoethanol was purchased from Sigma Chemical Co (St Louis, Missouri).

Introduction of 2-Pyridyl Disulfide Structures Into CTB or CB11 Peptide by N*-Succinimidyl 3-(2-Pyridyldithio)-Propionate.* Cholera toxin B subunit was purchased from List Biological Laboratory (Campbell, California). The process for creating CTB-protein conjugate has been described.[32] Briefly, 0.01 mL of *N*-succinimidyl 3-(2-pyridyldithio)-propionate (20 mg/mL in 99.5% ethanol) was added to 2 mg of CTB or CB11 in a volume of 2 mL in 0.1-mol/L sodium phosphate buffer (pH 7.5) containing 0.1-mol/L sodium chloride (NaCl). The mixture was incubated at 23°C for 30 minutes. The excess of the reagent was removed by gel filtration on a Sephadex G-25 column (Pharmacia Biotech, Piscataway, New Jersey). The content of 2-pyridyl disulfide structure in the modified protein or peptide was determined by adding 0.1 mL of dithiothreitol (DTT; 50 mmol/L) to 2.0 mL of protein solution. This treatment released pyridine-2-thione, which has a molecular absorptivity of 8.08×10^3 at 343 nm, from CTB or CB11. The amount of pyridine-2-thione released was equivalent to the content of 2-pyridyl disulfide groups in CTB or CB11. The modified CTB or CB11 was stored in 0.1-mol/L sodium phosphate buffer containing 0.1-mol/L NaCl, pH 7.5, at 4°C until used.

Preparation and Purification of CTB-CB11 Conjugate. The 2-pyridyl disulfide of CTB and CB11 was mixed and incubated overnight at room temperature in 0.1-mol/L sodium phosphate buffer (pH 7.5) containing 0.1-mol/L NaCl. The CTB-CB11 conjugate was purified through a Sephadex G-25 column and a nonreducing 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel. The conjugate above molecular weight 60 kd was cut out and eluted from the SDS-PAGE gel by elution buffer (100-mmol/L sodium bicarbonate, 3% sodium dodecyl sulfate [SDS], 8-mol/L urea, 25-mmol/L dithiothreitol) overnight at room temperature. The eluted conjugate was separated from the SDS-PAGE gel by a 0.45 Millex GV syringe filter (Millipore Co, Bedford, Massachusetts). Because SDS has been shown to inhibit the induction of immunity, deoxycholate coupled with a mixture of phosphatidylcholine, phosphatidylethanolamine, and cholesterol (50:45:5) was substituted for SDS in the purification process and dialyzed 3 days in 10-mmol/L Tris buffer (pH 7.4) to replace the detergent. The concentration of CTB-CB11 conjugate was determined in a 10% SDS-PAGE gel. The SDS, urea, dithiothreitol, phosphatidylcholine, phosphatidylethanolamine, and cholesterol were purchased from Sigma Chemical Co.

Induction of Autoimmune Diseases. The mice were divided into 5 groups (4 to 9 mice per group). The mice in group A were fed with phosphate-buffered saline solution (PBS; pH 7.4); these were the control mice. Group B received oral administration of 20 µg of CB11 peptide, group C received oral administration of 500 µg of CB11, and group D received oral administration of 20 µg of CTB-CB11. All groups received PBS or antigen 7 days before 200 µg of bovine type II collagen (in complete Freund's adjuvant) was administered for sensitization (day 0) at the base of the tail. A booster immunization of the same amount (in incomplete Freund's adjuvant) of antigen was given at the base of the tail on day 10 and ear-punched (approximately 2 mm) at the auricle of the right ear on day 12. The sera from all 4 groups of mice were collected on days 0, 11, and 25 after immunization. The development of chondritis was observed from days 11 to 24. The clinical score of chondritis was graded as 0 for mild erythema with little swelling due to the ear punch, 1 for moderate erythema and swelling of portions of the ear, and 2 for severe erythema and swelling of the entire ear.

T-Cell Proliferation Assay In Vitro. In groups A and D, draining lymph node cells were harvested from the inguinal, popliteal, and periaortic lymph nodes 10 days after the boost. For the T-cell proliferation assays, 1×10^6 T cells were cultured for 4 days with bovine CII (concentration ranging from 12.5 to 200 μg/mL) in the presence of 4×10^5 syngeneic irradiated spleen cells and then pulsed for 18 hours with 1 μCi of [^{3}H]-methylthymidine (Du Pont, Wilmington, Delaware) per well in 96-well plates. The incorporation of [^{3}H]-methylthymidine was measured by liquid scintillation counting. Values were expressed in the stimulation index as follows: [(cpm with antigen) – (cpm without antigen)]/(cpm without antigen). Each sample was run in triplicate.

Determining Bovine CII or CB Peptide–Specific IgG and Its Subclass. Blood from the mice in all 4 groups was collected, and the red blood cells were separated from sera by centrifugation. The bovine CII or CB peptide–specific IgG and its subclass were determined by enzyme-linked immunosorbent assay as follows. One hundred microliters of bovine CII or CB peptide (5 μg/mL in 0.1-mol/L carbonate buffer, pH 9.6) was dispensed in each well of a polystyrene microtiter plate (Costar, Cambridge, Massachusetts) and incubated overnight at 4°C. In the control assay, we coated a series of dilutions of normal mouse IgG (0 to 500 μg/mL; Sigma) on a 96-well plate. The antigen-coated plates were washed 3 times in 0.05% PBS–Tween 20 buffer and incubated with mice sera overnight at 4°C. The plates were washed 5 times with 0.05% PBS–Tween 20 buffer and incubated with ε-chain–specific anti-mouse IgG, IgG1, IgG2a, or IgG2b antibody (Sigma) overnight at 4°C. The plates were washed 5 times (0.05% PBS–Tween 20 buffer) before the addition of citric acid–phosphate buffer (pH 5.0) containing 0.15 mg/mL of *o*-phenylenediamine (Sigma). The color was developed at room temperature, and the reaction was stopped by 2.5-mol/L sulfuric acid. The color was measured at 492 nm (Bio-Rad, Richmond, California). The amount of IgG in each group was converted to micrograms per milliliter.

Measuring Cytokine mRNA Expression. The mice from groups A and D were painlessly sacrificed 10 days after the booster. T cells from the lymph nodes or Peyer's patches were removed and stimulated with bovine CII (150 μg/mL) or CB11 (100 μg/mL) in vitro for 4 hours. The cells were washed with PBS and messenger RNA (mRNA) was prepared (Biotecx, Houston, Texas). By means of murine leukemia virus reverse transcriptase (RT) and random hexanucleotide primer and the instructions of the Perkin Elmer Gene Amp RNA polymerase chain reaction (PCR) kit (Perkin Elmer, Branchberg, New Jersey), first-strand complementary DNA (cDNA) was generated from 1 μg of total RNA and subjected to RT-PCR analysis. To determine the relative abundance of each cytokine mRNA expression, we optimized the amount of each cDNA for PCR by the intensity of the amplified DNA products of β-actin from each RNA. In the PCR reaction mixture, one of the following was added at a final concentration of 0.2 μmol/L (all Clontech, Palo Alto, California): β-actin (5' primer, 5'-GTG GGC CGC TCT AGG CAC CAA-3'; 3' primer, 5'- CTC TTT GAT GTC ACG CAC GAT TTC-3') as a control primer, interleukin (IL)–2 (5' primer, 5'- TTC AAG CTC CAC TTC AAG CTC TAC AGC GGA AG -3'; 3' primer, 5'-GAC AGA AGG CTA TCC ATC TCC TCA GAA AGT CC-3'), IL-4 (5' primer, 5'-CAG CTA GTT GTC ATC CTG CTC TTC-3'; 3' primer, 5'-GTG ATG TGG ACT TGG ACT CAT TCA TGG-3'), IL-5 (5' primer, 5'-TGT CTG GGC CAC TGC CAT GGA GAT TC-3'; 3' primer, 5'-CCA TTG CCC ACT CTG TAC TCA TCA CAC-3'), IL-10 (5' primer, 5'-ATG CAG GAC TTT AAG GGT TAC TTG GGT-3'; 3' primer, 5'-ATT TCG GAG AGA GGT ACA AAC GAG G-3'), TGF-β (5' primer, 5'-TGG ACC GCA ACA ACG CCA TCT ATG AGA AAA CC-3'; 3' primer, 5'-TGG AGC TGC AGC AAT AGT TGG TAT CCA GGG CT-3'), or interferon (IFN)–γ primer (5' primer, 5'-TGC ATC TTG GCT TTG CAG CTC TTC CTC ATG GC-3'; 3' primer, 5'-TGG ACC TGT GGG TTG TTG ACC TCA AAC TTG GC-3'). The PCR condition was as follows: 200-μmol/L deoxy nucleotide triphosphate, 10-μCi [^{32}P] deoxy cytosine triphosphate, 50-μmol/L Tris–hydrochloric acid (pH 9.0), 50-μmol/L NaCl, 2-μmol/L magnesium chloride, 0.5-μmol/L DTT, and 2 units of Taq polymerase (Perkin Elmer) at a final volume of 20 μL. A negative control reaction was run with each sample to verify that no PCR bands appeared in the absence of template. The optimal amplification conditions were as follows: 45 seconds at 94°C for denaturation, 45 seconds for annealing, and 1 minute at 72°C for elongation. The annealing process was at 63°C for IL-2, IL-4, and transforming growth factor (TGF)-β and 65°C for β-actin, IFN-γ, IL-5, and IL-10. The PCR cycles were 30 for β-actin, IFN-γ, IL-5, and IL-10 and 35 for IL-2, IL-4, and TGF-γ. The amplified DNA was separated on a 5% PAGE gel. The amplified DNAs of β-actin, IL-2, IL-4, IL-5, IL-10, TGF-β, and IFN-γ had sizes of 540, 413, 354, 349, 455, 525, and 365 base pairs, respectively. The gel was dried on Whatman 3M paper and exposed to Kodak XAR film. In each electrophoresis run, intragel and intergel staining homogeneity was confirmed by the staining intensity of the molecular weight markers at both ends of the gels. In general, amplification kinetics was monitored for each PCR run by examining aliquots of the products on the gel. Amounts of the PCR products were compared during the cycle in which the amplification did not reach saturation.

Fluorescence-Activated Cell Sorter Analysis. The

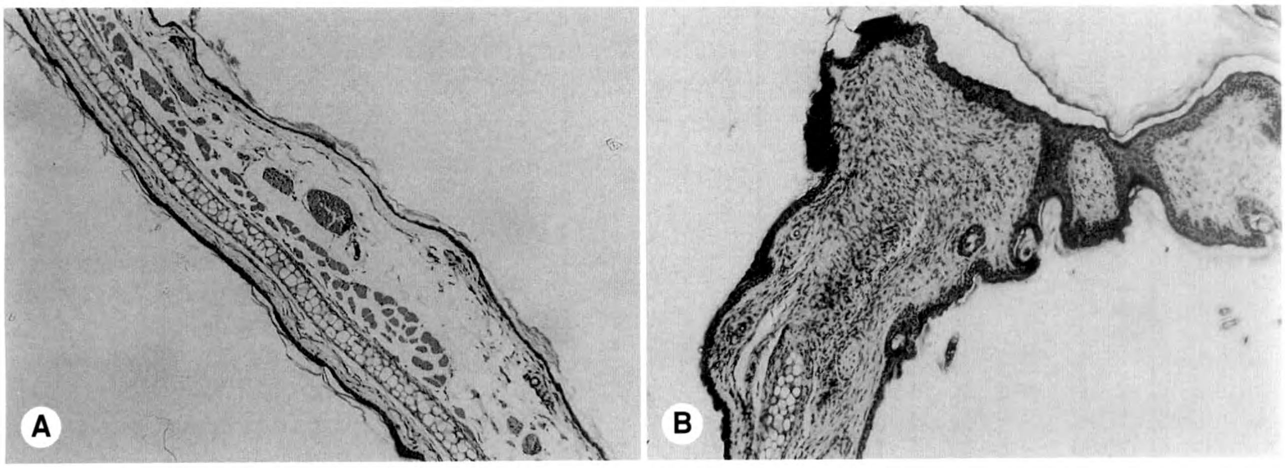

Fig 1. Auricular chondritis in mice. **A**) Control section. **B**) Inflammatory cells infiltrating cartilaginous layer.

T cells collected from lymph nodes (inguinal, popliteal, and periaortic areas) or Peyer's patches (1×10^6) of the control and experimental mice were washed twice with washing buffer (1X PBS, 5% fetal calf serum, 0.25% bovine serum albumin, and 0.02% sodium azide) and treated with 10-µg/mL CD8 monoclonal antibody (fluorescein isothiocyanate–labeled, PharMingen, San Diego, California) at 37°C for 30 minutes. The unlabeled CD8 antibody was washed once with washing buffer. The CD8-labeled T cells were then treated with CD4 monoclonal antibody (phycoerythrin-labeled, PharMingen) for 30 minutes at 37°C. The cells were washed with PBS solution, resuspended, and subjected to flow cytometric analysis on a flow cytometer at the University of Tennessee, Memphis (50H cytofluorograph; Ortho Diagnostic System Inc, Westwood, Massachusetts).

TABLE 1. CLINICAL SCORE AND INCIDENCE OF CHONDRITIS IN CONTROL MICE AND B10.RIII MICE ORALLY FED CTB-CB11

		Incidence of Chondritis	
*Oral Tolerogen**	*Clinical Score of Chondritis†*	*No.*	*%*
PBS	1.00 ± 0.00	4/4	100
20 µg of CB11	0.86 ± 0.69	5/7	71
500 µg of CB11	0.50 ± 0.55	3/6	50
20 µg of CTB-CB11	0.11 ± 0.33	1/9	11

*B10.RIII mice were fed single dose of phosphate-buffered saline solution (PBS), cyanogen bromide peptide 11 (CB11), or cholera toxin B (CTB)–CB11 conjugate 5 days before 2 injection immunizations with 200 mg of bovine collagen II. Mice were divided into 4 groups (4 to 9 mice per group).

†Left ears of sensitized mice were punched (approximately 2 mm deep). Mice were examined daily for development of chondritis from day 11 to day 24. Incidence presented here was based on day 18 observation after first bovine collagen II sensitization. Lesions were graded as 0 = mild erythema with little swelling due to ear punch, 1 = moderate erythema and swelling of portions of ear, 2 = severe erythema and swelling of entire ear. Data are presented as mean ± SD. Each clinical score of animals from 4 groups was statistically analyzed by Wilcoxon rank sum test. There is significant difference between group A and group D ($p < .05$).

Statistical Analyses. Statistical analysis was performed by a Wilcoxon rank sum test or an unpaired Student's *t*-test, as appropriate.

RESULTS

Oral Administration of CTB-CB11 Conjugate and Protection From Chondritis. When mice were fed with PBS and challenged with bovine type II collagen, all of the mice (group A; 4 of 4) developed chondritis with moderate erythema and swelling (Fig 1). When mice were fed with 20 µg of CB11 (group B) or 500 µg of CB11 (group C), 5 of 7 (71%) or 3 of 6 mice (50%) developed chondritis with moderate erythema and swelling. The average clinical scores of chondritis decreased after oral administration of CB11. Only 1 of 9 mice (11%) in the group fed 20 µg of CTB-CB11 (group D) developed chondritis with moderate erythema and swelling (Fig 1A). The clinical score of this CTB-CB11–fed group of mice was significantly reduced (Table 1). There was a statistically significant difference between groups A and D in the clinical score ($p < .05$), but not between the other groups. In order to check whether oral administration of CTB effects the development of chondritis, we orally fed mice with 2 or 13 µg of CTB. When mice were fed with 2 or 13 µg of CTB alone, 6 of 6 mice in each group developed chondritis with moderate erythema and swelling similar to that of control group mice. These results suggest that oral administration of a small amount of CB11 (20 µg) provides protection from developing chondritis. Oral administration of a high dosage of CB11 (500 µg) provided better protection. A single administration of CTB-CB11 conjugate (20 µg) before challenge was sufficient to induce tolerance to the development of chondritis.

T-Cell Responses in Controls and Mice Orally Fed CTB-CB11. T cells harvested from the lymph nodes of control and experimental mice showed a similar

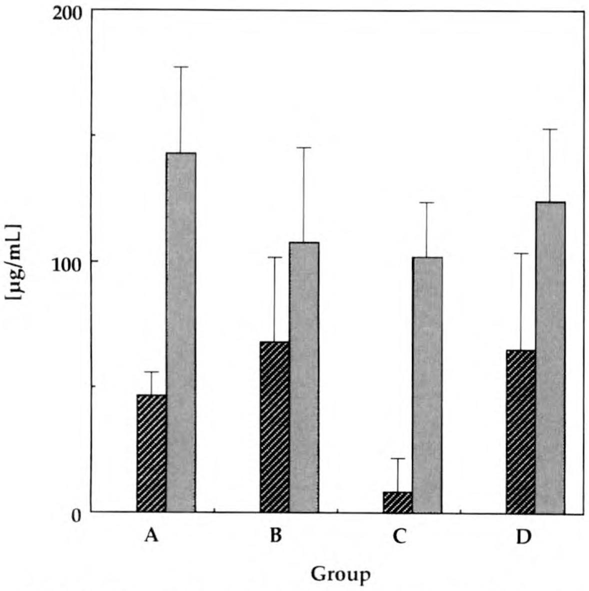

Fig 2. Determination of type II collagen–specific IgG and its subclasses. B10.RIII mice were divided into 4 groups. Group A are control mice that were orally fed with phosphate-buffered saline solution. Groups B, C, and D were fed orally with 20 µg of cyanogen bromide peptide 11 (CB11), 500 µg of CB11, and 20 µg of cholera toxin B–CB11 conjugate, respectively. Each group had 4 to 9 mice. Sera were collected 6 times after first immunization with bovine collagen II. Levels of bovine collagen II–specific IgG were measured by standard methods. Data are presented as mean ± SD. There was statistically significant difference between group A and group C at day 11 (p < .005). Hatched bars — day 11; solid bars — day 26.

T-cell response in vitro when the bovine CII concentration ranged from 12.5 to 100 µg/mL. However, T cells from the mice fed CTB-CB11 showed a higher response at 200 µg/mL of bovine CII (Fig 2). These results suggest that both control and CTB-CB11 conjugate–fed mice developed T-cell proliferative responses to bovine CII in vitro.

Bovine CII–Specific IgG Production in Controls and in Mice Orally Fed CB11 or CTB-CB11 Conjugate. We examined bovine CII–specific IgG responses from control and experimental mice for a period of 26 days. In groups B and D, IgG production was higher than in group A on day 11, and then showed a level similar to that of control mice on day 26. But, while the production of IgG in group C was much lower at the initial stage (on day 11), production significantly increased on day 26. There was a statistically significant difference between groups A and C at day 11 (p < .005). The patterns of CII-specific IgG subclasses (IgG1, IgG2a, and IgG2b) in all 4 groups of mice were similar to the pattern of total IgG production (data not shown). These results suggest that high doses of orally fed CB11 suppressed IgG production at the initial stage but later substantially increased production to reach levels similar to those seen in the other tolerogen-fed mice (Fig 2). Also, low-dose CB11–fed mice, whether the CB11 is CTB-conjugated or not, have higher CB11-specific antibody levels, whereas the high-dose–fed mice have a transient decrease in antibody levels.

CB Peptide–Specific Antibody Responses in Controls and in Mice Fed CB11 or CTB-CB11 Conjugate. For an observation period of 26 days, no significant production of CB8-, CB9-, or CB10-specific antibodies was detected in the sera from the 4 groups of mice. In the CB11-specific antibody responses, orally feeding 500 µg of CB11 suppressed CB11-specific antibody production at day 11 and significantly increased production on day 26. All of the mice

TABLE 2. RELATIVE LEVELS OF ANTIBODY TO EACH CYANOGEN BROMIDE PEPTIDE OF TYPE II COLLAGEN

*Day**	*Group†*	*Bovine Collagen II*	*CB8*	*CB9*	*CB10*	*CB11*
0	A	0.020 ± 0.008	0.027 ± 0.007	0.027 ± 0.014	0.015 ± 0.010	0.041 ± 0.004
	B	0.038 ± 0.013	0.011 ± 0.006	0.026 ± 0.016	0.017 ± 0.008	0.055 ± 0.012
	C	0.026 ± 0.003	0.014 ± 0.000	0.070 ± 0.014	0.016 ± 0.006	0.044 ± 0.009
	D	0.061 ± 0.015	0.032 ± 0.021	0.035 ± 0.022	0.046 ± 0.013	0.075 ± 0.017
11	A	0.301 ± 0.011	0.013 ± 0.000	0.003 ± 0.000	0.019 ± 0.006	0.145 ± 0.021
	B	0.472 ± 0.024	0.027 ± 0.000	0.000 ± 0.000	0.036 ± 0.020	0.316 ± 0.007
	C	0.117 ± 0.008	0.021 ± 0.011	0.018 ± 0.008	0.045 ± 0.025	0.099 ± 0.013
	D	0.457 ± 0.003	0.048 ± 0.020	0.015 ± 0.016	0.093 ± 0.013	0.299 ± 0.023
25	A	0.527 ± 0.005	0.091 ± 0.012	0.023 ± 0.012	0.095 ± 0.012	0.318 ± 0.011
	B	0.556 ± 0.011	0.114 ± 0.016	0.054 ± 0.023	0.115 ± 0.026	0.468 ± 0.003
	C	0.475 ± 0.016	0.075 ± 0.008	0.024 ± 0.015	0.106 ± 0.032	0.384 ± 0.006
	D	0.507 ± 0.021	0.084 ± 0.008	0.029 ± 0.016	0.072 ± 0.033	0.422 ± 0.004

Numbers of antibodies are shown by optical density values at 492 nm. Serum samples from each group were pooled and tested in triplicate. Data are shown as mean ± SD.

*IgG antibody titers to bovine collagen II and cyanogen bromide peptides 8, 9, 10, and 11 were determined by enzyme-linked immunosorbent assay using sera collection at day 0 (first immunization), day 11, and day 25.

†B10.RIII mice were divided into 4 groups. Group A is control group orally fed phosphate-buffered saline solution. Groups B, C, and D were orally fed 20 µg of CB11, 500 µg of CB11, or 20 µg of cholera toxin B–CB11 conjugate.

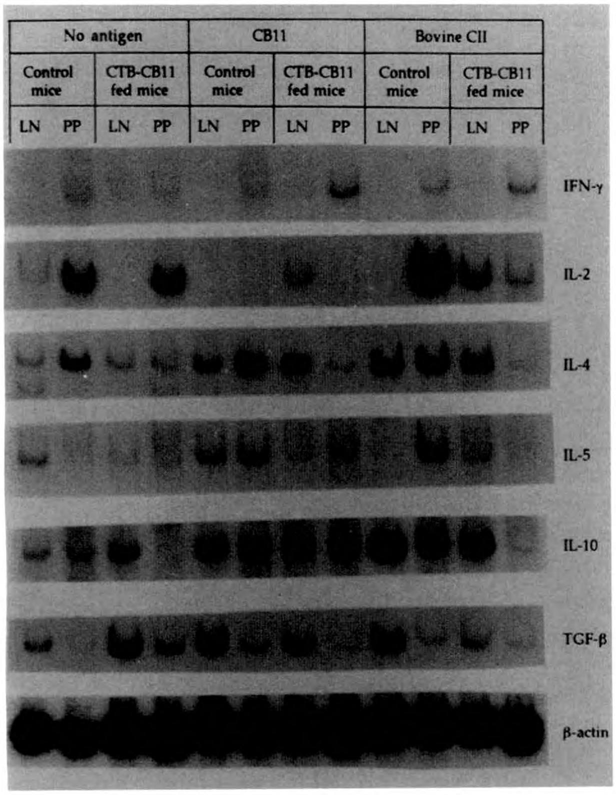

Fig 3. Cytokine gene expression in controls and mice orally fed cholera toxin B (CTB)–CB11 conjugate. T cells were collected from lymph nodes (LN) and Peyer's patches (PP) 10 days after boost and cultured in presence of no antigen (control), bovine collagen II (CII; 150 µg/mL), or CB11 (100 µg/mL) for 4 hours. Cells were harvested and total RNA was prepared. First-strand complementary DNA was synthesized from 1 µg of total RNA by means of Moloney murine leukemia virus reverse transcriptase and random hexamer according to instructions of Perkin Elmer Gene Amp RNA polymerase chain reaction kit. Reverse transcriptase–polymerase chain reactions were done with cDNA with different primers specific for interferon (IFN)–γ, interleukin (IL)–2, IL-4, IL-5, IL-10, transforming growth factor (TGF)–β, and β-actin.

that received 20 µg of PBS solution and CTB-CB11 conjugate produced CB11-specific antibodies from day 11 to day 26 (Table 2). These results suggest that mice receiving a high oral dose of CB11 peptide suppressed CB11 antibody production in the early stage. However, a higher titer of CB11 antibody production emerged in the late stage. Mice orally fed CTB-CB11 conjugate did not diminish CB11 antibody secretion during the 26 days. These data indicate that group A and D mice developed antibodies to bovine CII with similar fine specificity.

Cytokine Production of T Cells From Lymph Nodes and Peyer's Patches. When T cells were harvested from Peyer's patches of group D mice and stimulated with CB11 or bovine CII in vitro, a higher mRNA expression of IFN-γ and nonsignificant levels of IL-2, IL-4, IL-5, IL-10, and TGF-β were detected, in comparison with control mice. However, T cells from the inguinal, popliteal, and periaortic lymph nodes of these mice showed an increase of IL-2 and IL-4 accompanying a decrease of IL-10 and TGF-β (Fig 3). These data indicate that a single administration of a low dose of CTB-CB11 induced TH1 cytokine in Peyer's patches.

DISCUSSION

Studies of oral administration of antigen to animals provide at least 2 explanations for the tolerance mechanism. Feeding with a large dose of antigen favors anergy and deletion of T cells, while multiple small doses of antigen favor the development of regulatory T cells.[33] Antigen-driven bystander suppression also has been demonstrated in several autoimmune animal models, such as experimental allergic encephalomyelitis and collagen-induced arthritis.[34,35]

A cyanogen bromide peptide of bovine CII, CB11 has been identified as one of the major T-cell epitopes in collagen-induced autoimmune diseases.[29,36,37] Even though oral induction of tolerance has been successfully applied in autoimmune diseases, one of the potential problems that limits its therapeutic application in patients is the large dose and continuous administration of antigens needed. Therefore, an effective antigen delivery system is necessary for further application of oral induction of tolerance. Cholera toxin B has been reported as an effective transmucosal antigen carrier in several tolerance studies.[6,17,38,39] In our study, we chose to orally administer CTB-conjugated CB11 before sensitizing the animals to bovine CII. The results clearly suggest that CTB-CB11 conjugate induced tolerance to type II collagen in chondritis (Table 1). A single oral administration of CB11 before immunization with CII could suppress chondritis in B10.RIII mice. Cholera toxin B provided an excellent delivery system to protect against chondritis by administering a minute amount of CTB-CB11; the incidence of chondritis was reduced to 11%.

It is recognized that collagen-induced chondritis is mediated by cellular and humoral responses. Oral administration of 500 µg of CB11 showed a decrease in the production of bovine CII–specific IgG and its subclasses at an early stage (Fig 2). The diminution of antigen-specific immunoglobulins may contribute to the suppression of chondritis (50%) at the early stage. It is interesting to note that an increase of antigen-specific IgG and its subclasses was seen at the late stage and reached levels similar to those in the control mice. Similar observations were reported in earlier studies.[40,41] When CB11 peptide of bovine CII was used as a tolerogen in B10.RIII mice, antibodies to bovine CII in CB11-tolerant mice showed levels similar to those in control mice.[42] Also, CB11 generated a weaker T cell response than CB10 in B10.RIII (H-2^r), which was different from the prominent T cell responses to CB11 in DBA/1 and B10.Q (H-2^q) mice.[30,43-45] However, mice fed a minute amount of CTB-CB11 showed higher levels of IgG and its subclasses at day 11, even though chondritis was protected by oral administration. These results suggest that the mechanism for suppressing chondritis in group D is distinct from that in group C.

When T cells were stimulated with antigens in vitro, group D showed a pattern similar to that seen in group A (data not shown). Epitope mapping studies indicate that the group D mice developed antibodies to bovine CII with fine specificities similar to those seen in group A (Table 2). These results suggest that a single oral administration of CTB-conjugated CB11 does not alter the stimulation or the recognition mechanism of T or B cells.

The increased mRNA expression of IFN-γ and the diminution of IL-4, IL-5, and IL-10 in group D suggest that this oral administration process induced TH1 cells rather than TH2 cells in Peyer's patches at the early stage (Fig 3). The induction of regulatory T cells in gut-associated lymphoid tissue has been reported to mediate a suppression mechanism.[40,46] This suppression mechanism favors the induction of TH2 cells and T cells that secrete TGF-β. However, an increased IFN-γ secretion in Peyer's patches has been reported in oral administration of high doses of ovalbumin in ovalbumin T cell receptor–transgenic mice.[47] Also, the fact that oral administration of low doses of CTB–myelin basic protein promoted IFN-γ production has been seen in the draining popliteal lymph nodes of mice with experimental allergic encephalomyelitis.[6] A human study has reported that the oral administration of vaccines containing CTB elevated IFN-γ production in the gut.[48] It is of interest to note that IFN-γ can induce T cells to release active TGF-β.[49] However, the increased mRNA expression of IL-2 and IL-4 in group D suggests that both TH1 and TH2 cells were activated in the inguinal, popliteal, and periaortic lymph nodes (Fig 3). Complex manifestations of an evolving T-cell response (TH1 and TH2) were also seen in oral feeding of ovalbumin.[50] Obviously, the tolerance mechanism produced through oral administration with antigen is not as simple as the shift of TH1 to TH2 cells to provide protection. These results suggest that the protection from autoimmune diseases produced by oral administration of CTB-conjugated antigen is distinct from the protection provided by conventional oral tolerance induction.

Our results presented here suggest that CTB provided a strong delivery system for oral administration, and that feeding of a minute amount of CTB-conjugated CB11 could prevent collagen-induced chondritis in mice by a mechanism that is distinctly different from that of conventional oral tolerance induction.

ACKNOWLEDGMENTS — The authors thank Dr Hiroshi Matsuoka for the tissue work, Dr Hyok-yop Lee for statistics, and Dr G. J. Thobecke, Dr A. Postlethwaite, and Dr David Ambruster for reading the manuscript and making helpful suggestions.

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MANAGEMENT OF THE TINNITUS PATIENT

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Ann Otol Rhinol Laryngol 110:2001

MYELINATION OF THE HUMAN AUDITORY NERVE: DIFFERENT TIME COURSES FOR SCHWANN CELL AND GLIAL MYELIN

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The goal of this study was to trace the development of myelin in the human auditory nerve. To do this, we used the Woelcke iron-hematoxylin technique to stain myelin sheaths in sections from fetal temporal bones and brain stems. In the cochlea, aggregations of Schwann cells are seen in the modiolus and along the spiral lamina by the 15th fetal week. By the 22nd fetal week, dense arrays of stained Schwann cells are present on auditory nerve axons within the temporal bone. By the 24th fetal week, light myelin sheaths extend up to, but not beyond, the glial junction. Myelin sheaths are not present in the auditory nerve central to the glial junction until the 26th fetal week or later. These results demonstrate a gap of several weeks between the onset of Schwann cell myelination distally and glial myelination proximally. The period between these two events may represent the time of final maturation of the organ of Corti.

KEY WORDS — auditory nerve, cochlea, myelin, oligodendrocyte, Schwann cell.

INTRODUCTION

The auditory nerve, like all peripheral nerves, is covered by two types of myelin. The distal portion of the nerve within the temporal bone is ensheathed in myelin formed by Schwann cells derived from the neural crest, while axons in the central (intradural) portion of the nerve are surrounded by myelin formed by oligodendrocytes derived from the neural tube. The point of transition from Schwann cell to glial myelin, known as the glial junction, is located at the level of the internal auditory canal (porus acusticus). In species such as the cat, the auditory nerve has a total length of about 3 mm,[1] and the peripheral and central segments of the nerve are almost equal in length. In adult humans, however, the auditory nerve is 20 to 30 mm in length, and most of the nerve is covered by glial myelin.

The appearance of myelin is an important event in the developmental process because of its close relationship to the onset of rapid and synchronized axonal conduction. However, something of a discrepancy exists in the descriptions of early development of the human auditory system. On the one hand, studies of fetal cochlear development have reported a considerable degree of maturation by the 20th to 22nd weeks of gestation. At this age, the intraepithelial fluid spaces of the organ of Corti (tunnel of Corti and spaces of Nuël) are well developed in all but the apical coil.[2-5] Both inner and outer hair cells have stereocilia bundles that are acquiring their characteristic shape, although a kinocilium is still present.[3-7] Hair cell afferent innervation is also relatively mature, with nerve fibers present in the tunnel of Corti and between hair cells, and afferent synaptic endings in proximity to both inner and outer hair cells.[2,4,5,7-9] Pujol and Uziel[10] have argued cogently that the human cochlea at the 20th fetal week is at a stage of maturation equivalent to that of other mammalian species at the time of their first response to sound.

On the other hand, anatomic and physiological studies of the human brain stem suggest a somewhat later onset of function. In our laboratory, we observe the first signs of myelination in the proximal auditory nerve and brain stem pathway at the 26th to 29th gestational weeks.[11] This is consistent with the first appearance of recordable evoked brain stem responses in premature infants at 28 to 29 weeks' gestational age.[12,13] In addition, consistent startle responses to sound appear in human fetuses predominantly between the 26th and 28th weeks of gestation.[14,15] Taken together, these observations suggest that rapid conduction through brain stem pathways does not begin until the 26th fetal week or later.

The goal of the present study was to resolve this discrepancy by investigating the process of myelination in the entire auditory nerve. In carrying out this study, we used material from our archival collections of human fetal temporal bones and brain stems. The same histologic staining method for myelin was used in celloidin sections from both cochleas and brain stems. Although our goal was to establish the timepoint at which myelination occurred across the entire auditory nerve, we appear instead to have produced evidence for a two-stage process, with myeli-

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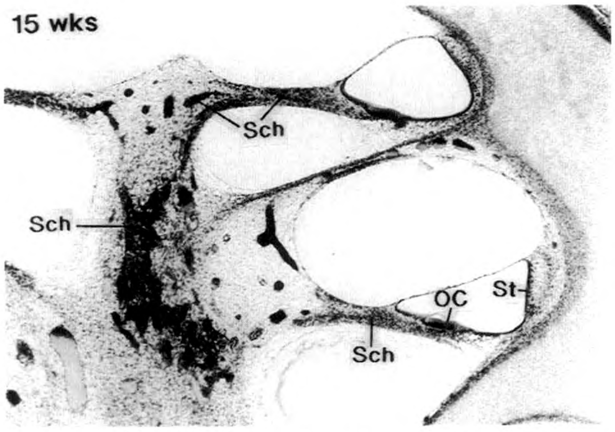

Fig 1. Low-power view of cochlea at 15th fetal week (original ×10). Clusters of darkly staining Schwann cells (Sch) are present in modiolus, and bands of stained Schwann cells extend along spiral lamina. OC — organ of Corti, St — stria vascularis.

nation of the distal segment of the nerve occurring a few weeks earlier than myelination of the central segment.

MATERIALS AND METHODS

For the study of development of the intracochlear portion of the nerve, we used sections from human fetal cochleas at 15, 22, 33, 38, and 40 weeks of gestation that were archival material in the House Ear Institute temporal bone bank. For the study of the proximal portion of the nerve, we used sections from human fetal brain stems at 16, 22, 24, 26, 29, 33, and 40 weeks of gestation that had been obtained within the past 8 years at neuropathological autopsy at the University of Southern California–Los Angeles County Hospital.

Both the brain stems and the temporal bones had been initially preserved in buffered formalin solution for several weeks. The temporal bones had been decalcified by immersion for several weeks in a 0.27-mol/L solution of ethylenediamine-tetraacetic acid (EDTA) in 1% formalin. At the end of the decalcification procedure, the EDTA was removed by passing the tissue through several changes of phosphate buffer, pH 7.4. Both the brain stem tissue blocks and the decalcified temporal bones were dehydrated in graded ethanols and infiltrated with solutions of celloidin (Parlodion) increasing in several steps to 12% to 15%. The tissue blocks were then embedded in 12% to 15% celloidin. The celloidin blocks were sectioned on a sliding microtome, the cochleas at a thickness of 20 μm and the brain stems at 40 μm. All sections were preserved in numbered sequence on tracing paper in 80% ethanol. The advantage of celloidin embedding in preserving tissue morphology is illustrated by the fact that excellent staining was obtained in all sections, including temporal bone specimens that had been in storage for more than four decades.

Staining of sections for this study was done by the Woelcke iron-hematoxylin method, a technique that we have found to be sensitive during all stages of human neural development. Visualization of myelin sheaths and reactive glial cells is obtained, despite loss of lipids during the embedding process, presumably because of staining of the remaining glycoprotein components of the myelin sheath. These proteinaceous components include such elements as MBP (myelin basic protein) and MAG (myelin-associated glycoprotein). In the Woelcke procedure, celloidin sections were mordanted overnight in 2.5% ferric ammonium sulfate and then stained in a solution of 1% aged alcoholic hematoxylin and 0.7% saturated lithium carbonate. The sections were rinsed in distilled water and dehydrated in graded ethanols. At 100% concentration, ethyl alcohol was replaced by butyl alcohol to avoid softening of the celloidin. The sections were then cleared in xylene and mounted with Permount. Stained sections were examined and photographed on a Nikon Optiphot. Comparison series of every eighth section stained for cytoarchitecture were available for all specimens. In these series, the temporal bone sections were stained with hematoxylin and eosin, and the brain stems were stained with cresylecht violet (Nissl method).

RESULTS

Fetal Week 15. In the fetal cochlea at 15 weeks (Fig 1), the cochlear capsule is still entirely cartilaginous, in contrast to the rest of the skull, which has begun to show areas of ossification. Within the scala media, the developing organ of Corti rests on a thick cellular layer that will eventually thin and become the basilar membrane. At this stage, the organ of Corti is a solid cellular mass lacking the intraepithelial fluid spaces (tunnel of Corti and spaces of Nuël). Above the organ of Corti, a thin tectorial membrane is attached to the limbus. The developing stria vascularis is seen as a single layer of cells on the surface of the spiral ligament. A prominent feature at the 15th fetal week is clusters of darkly stained Schwann cells in the modiolus. Similar stained cells spread along the spiral lamina as far as the organ of Corti. In the brain stem at a comparable age (16th fetal week), there are no aggregations of stained glial cells in the auditory nerve.

Fetal Week 22. In the cochlea at the 22nd fetal week (Fig 2), the scala media shows some signs of maturation. Reissner's membrane is thicker, the tissue supporting the organ of Corti is thinner, and the

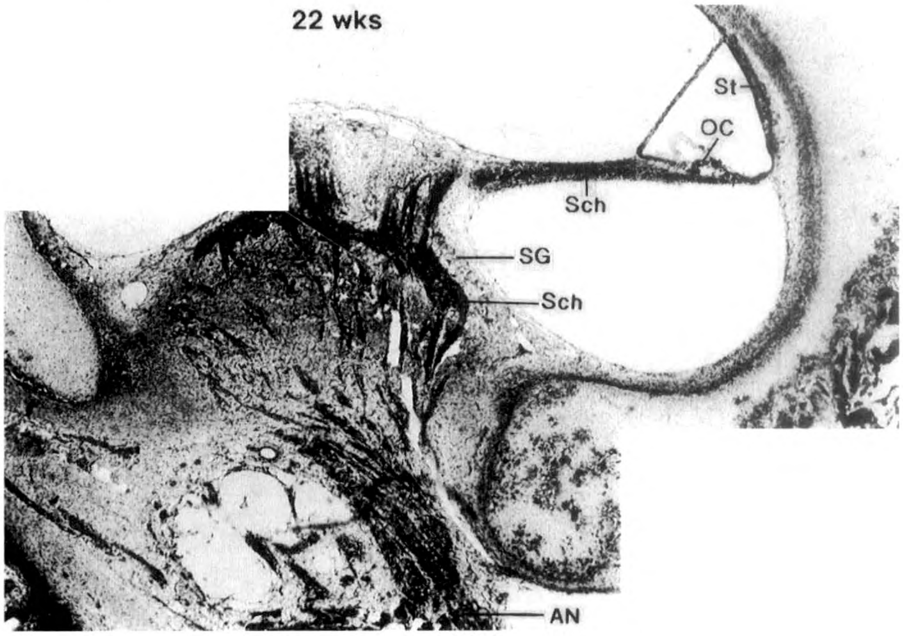

Fig 2. Low-power view of cochlea at 22nd fetal week (original ×10). Stained Schwann cells (Sch) outline fascicles of axons in modiolus and along osseous spiral lamina. AN — auditory nerve, OC — organ of Corti, SG — spiral ganglion, St — stria vascularis.

tectorial membrane is better developed than at 15 weeks. On the surface of the spiral ligament, the stria vascularis now consists of distinct marginal, intermediate, and basal cell layers. The organ of Corti in this specimen has suffered artifactual damage, but it is apparent that a tunnel of Corti now separates inner hair cells from outer hair cells.

Within the modiolus, darkly stained Schwann cells now outline the fascicles of axons that are coalescing to form the auditory nerve (Fig 2). The band of Schwann cells in the osseous spiral lamina is denser than at 15 weeks (Fig 3), and faintly staining myelin sheaths are just visible distally, beneath the scala media. The distal auditory nerve shows a dense concentration of stained Schwann cells (Fig 4A), and at higher power the spiraling fascicles of axons are seen to be outlined by linear arrays of Schwann cells (Fig 4B). In contrast, in the brain stem at this age, there is no accumulation of stained oligodendrocytes (myelination gliosis) in the auditory nerve or any of the central pathways.

Fetal Week 24. A brain stem section at 24 weeks of gestation (Fig 5A) passes through the ventral cochlear nucleus and cerebellar flocculus. The auditory nerve at this level enters the ventral cochlear nucleus, forming the nerve root. The nerve in this specimen includes its entire central segment and a portion of the distal segment that was avulsed from the temporal bone. In the distal portion of the nerve (Fig 5B), light myelin sheaths extend up to, but not beyond, the glial junction. The adjacent proximal portion of the nerve exhibits a heavy concentration of stained oligodendrocytes, but no myelin sheaths are visible.

Fetal Week 26. A brain stem specimen from a slightly more advanced stage, the 26th fetal week (Fig 5C), includes the ventral cochlear nucleus and the auditory nerve entering the nucleus. The vestibular nerve root is located medial to the cochlear nucleus. In a higher-power view of the auditory nerve (Fig 5D), axons entering the ventral cochlear nucleus are surrounded by stained glial cells and have light myelin sheaths. This is the youngest case in our series with detectable myelin in the proximal auditory nerve.

Fetal Week 33. At 33 weeks, axons are clearly myelinated in the segment of the nerve distal to the glial junction (Fig 6A). In brain stem sections from the same fetal age, axons at their point of entry into the ventral cochlear nucleus (Fig 6B) show a degree of myelination similar to that in the distal end of the nerve. At this age, the density of stained cells in both segments of the nerve is less than at earlier developmental stages. At later ages, ie, at 38 and 40 fetal weeks (not illustrated), the intensity of staining is not obviously different from that seen at 33 weeks.

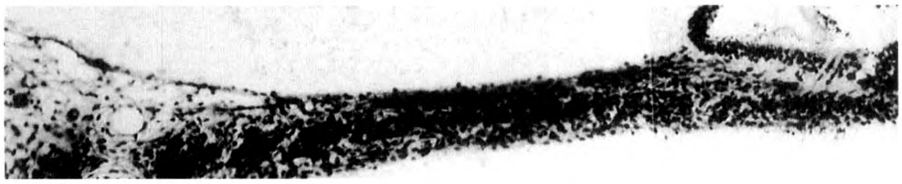

Fig 3. Higher-power view of osseous spiral lamina at 22nd fetal week (original ×25). Darkly stained Schwann cells are distributed along axons, and faint myelin sheaths are visible at distal end beneath scala media.

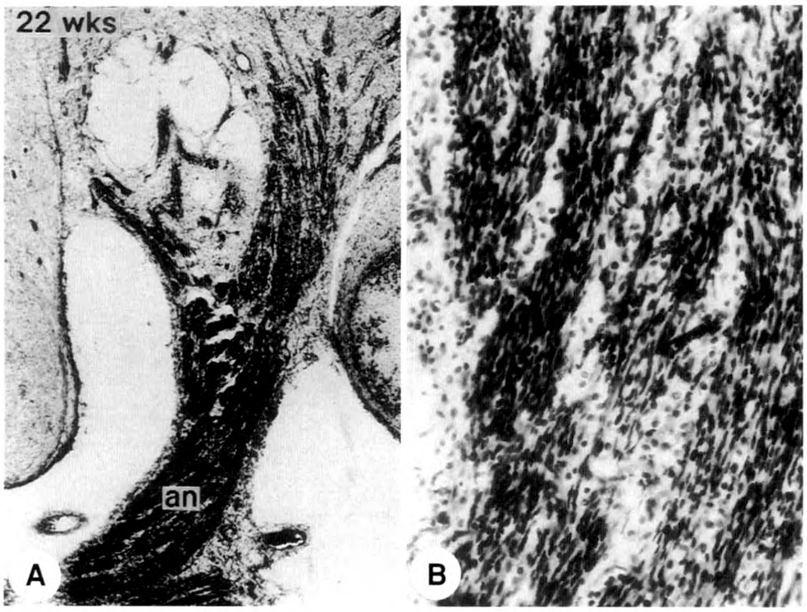

Fig 4. Auditory nerve at 22nd fetal week. **A)** In low-power view, nerve (an) shows dense accumulation of stained Schwann cells (original ×25). **B)** At higher power, spiraling fascicles of axons in nerve show linear arrays of stained Schwann cells (original ×50).

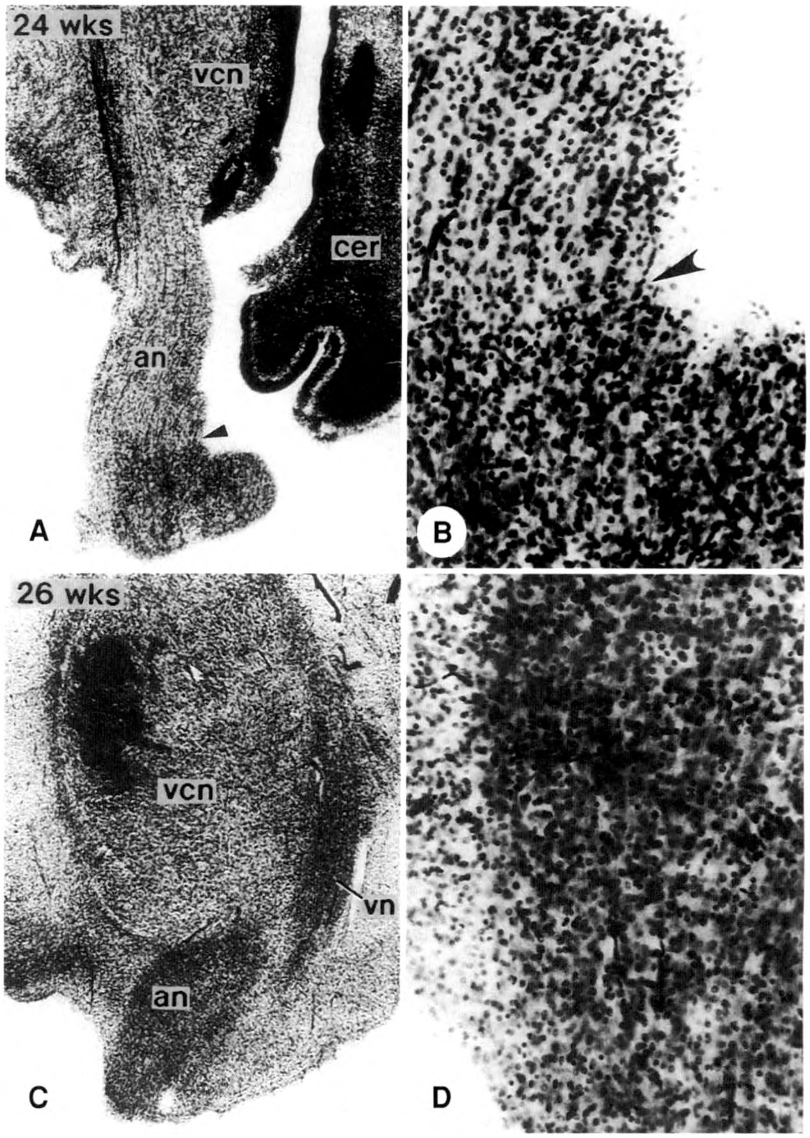

Fig 5. Auditory nerves at **A,B)** 24th and **C,D)** 26th fetal weeks. **A)** Brain stem section at 24th fetal week passes through ventral cochlear nucleus (vcn) and cerebellar flocculus (cer; original ×25). Arrowhead — glial junction of auditory nerve (an). **B)** In higher-power view of nerve, lightly myelinated axons are present distal to glial junction (arrowhead), but not proximal to it (original ×50). **C)** Brain stem section at 26th fetal week includes auditory nerve (an) entering ventral cochlear nucleus (vcn; original ×25). Vestibular nerve (vn) passes medial to nucleus. Densely cellular region within cochlear nucleus is area of angiogenesis. **D)** At higher power, auditory nerve axons entering ventral cochlear nucleus are surrounded by numerous oligodendrocytes and have light myelin sheaths (original ×50).

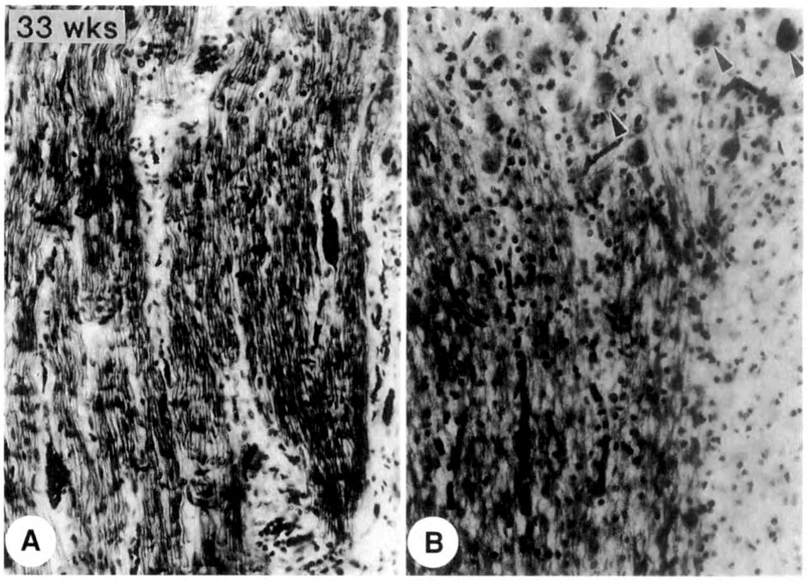

Fig 6. Auditory nerve at 33rd fetal week (original ×50). **A**) Axons in distal segment of nerve are myelinated, but no longer show arrays of stained Schwann cells. **B**) Axons at point of entry of nerve into brain stem show degree of myelination equivalent to that in distal segment. Arrowheads — neurons in ventral cochlear nucleus.

DISCUSSION

The present observations indicate that auditory nerve myelination occurs in 2 stages, with Schwann cell myelin present by the 22nd to 24th fetal weeks, but myelination not occurring in the proximal nerve until the 26th week or later. These findings are in agreement with a previous observation of myelinated fibers within the osseous spiral lamina in a fetus at 22 weeks of gestation,[2] but there are no comparable ultrastructural studies of human central pathways. It is difficult to know whether a similar two-stage process occurs in other mammalian species, given the more compressed time scale of their development and the scant information available. In kittens at the time of birth, intracochlear axons have 10 to 20 lamellae of myelin.[16] At the same age, axons in the proximal auditory nerve have only 10 lamellae of myelin.[17] More information would be necessary to judge whether this reflects different stages of maturation in the proximal and distal segments of the nerve.

Supporting evidence for a two-stage myelination process is provided by developmental studies of the auditory brain stem response (ABR). Intrasurgical recordings in humans locate the generation sites of ABR waves I and II in the auditory periphery[18,19] and indicate that the wave I-II interval represents distal nerve conduction time. This assumption is supported by dipole studies[20] indicating generation of dipole I at the distal end of the auditory nerve and generation of wave II by passage of nerve impulses through the porus. Intrasurgical recordings have also localized the site of generation of later ABR waves. A potential contemporaneous with wave III of the scalp record was recorded over the cochlear nuclei,[18] whereas electrodes placed higher on the pons recorded potentials corresponding to scalp-recorded waves IV and V.[18,19] Analysis indicates that the III-IV interwave interval represents purely central axonal conduction.[21] In early development of the ABR, differential maturation of the I-II and III-IV interwave intervals is observed.[21-23] The duration of the I-II interval is adult-like even in the youngest infant group studied, those at 29 to 34 weeks of gestation. In contrast, the III-IV interval does not reach an adult value until around the time of term birth, at 38 to 42 weeks' gestational age. Because speed of axonal conduction is directly related to degree of myelination, these findings imply earlier maturation of myelin in the distal segment of the auditory nerve than in the brain stem pathway.

As pointed out by Pujol and Uziel,[10] the human organ of Corti at the 20th fetal week, with its open intraepithelial spaces, free tectorial membrane, and mature inner hair cell innervation, has the status of the organ in other species at the time of onset of cochlear function. What occurs in the human cochlea in the time between the onset of myelination distally at 22 fetal weeks and the onset of myelination centrally at around 26 weeks? During this period, the major developmental changes occur in outer hair cells and their innervation, and in the basilar membrane. Outer hair cells and their supporting cells attain their adult elongated shape. The synaptic pattern on the outer hair cells changes, with enlargement of the efferent endings and retraction of the afferent terminals. The basilar membrane thins and develops its filamentous structure. These morphological changes should result in major changes in the micromechanical properties of the cochlea. By analogy with other mammalian species, functional changes in this period should include increasing cochlear sensitivity, the development of frequency selectivity, and the appearance of nonlinear characteristics of the amplitude-intensity function.[10] Later myelination of the central auditory system would delay signal transmission until these peripheral changes had occurred.

At present there is not a complete picture of the mechanisms of myelination formation. The process is known to involve intrinsic factors such as interactions between axons and glial cells. For example, the presence of axons is necessary for normal expression of Schwann cell myelin-specific genes and messenger RNAs[24] and for transfer of myelin proteins into the Schwann cell membrane.[25] In the central nervous system, electrical activity in axons induces proliferation of oligodendrocyte precursor cells[26] and enhances myelinogenesis.[27] However, it is not clear how electrical activity in a peripheral nerve could have a differential effect on myelin-producing cells on adjacent segments of the same axon.

Extrinsic factors also play a role in control of myelination. Thyroid hormone affects glial gene expression in both the distal and proximal segments of the auditory nerve,[28] and there are factors that might differentially influence the timing of Schwann cell and glial myelin formation. For example, early Schwann cell development is regulated by *neu*-differentiation factor, also known as glial growth factor.[29] In the central nervous system, a complex of factors, including insulin-like growth factor, interact to control development of oligodendrocytes.[30] Certainly, central and peripheral myelin are differentially affected by disease and the effects of genetic mutations. Still, we are not able to definitively identify the mechanisms that enable a single axon to promote the formation of two types of myelin, to create and maintain the precisely located border between them, and to control the time courses of their development.

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Ann Otol Rhinol Laryngol 110:2001

ACUTE OTITIS MEDIA IN BRAZILIAN CHILDREN: ANALYSIS OF MICROBIOLOGY AND ANTIMICROBIAL SUSCEPTIBILITY

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Between 1990 and 1995, a total of 300 children, ages 2 months to 5 years, received diagnoses of acute otitis media (AOM) in a hospital emergency room in São Paulo, Brazil, and were recruited for this study. The investigation was undertaken, first, to identify microorganisms and antimicrobial susceptibilities of pathogens from AOM in Brazilian children; next, to ascertain, by comparison, whether the isolates of *Streptococcus pneumoniae* have the same serotypes as those included in the new conjugated heptavalent pneumococcal vaccine; and last, to determine whether additional and/or different serotypes are needed in the vaccine to ensure an immunogenic response against pneumococcal pathogens for the indigenous children in this study. Microorganisms were isolated from ear fluid of 192 patients (64%). The 5 most prevalent pathogens were *S pneumoniae* (48 isolates; 16%), *Haemophilus influenzae* (21 isolates; 7%), *Moraxella catarrhalis* (15 isolates; 5%), *Pseudomonas aeruginosa* (6 isolates; 2%), and *Staphylococcus aureus* (3 isolates; 1%). These 5 represented 93 of the 192 total isolates. Resistance to antibiotics was found in the 3 primary pathogens. No high-level resistance of *S pneumoniae* to penicillin was found; instead, there was high-level resistance to trimethoprim-sulfamethoxazole. Ten serotypes of *S pneumoniae* were isolated: 6B, 9V, 11A, 16, 18C, 19A, 19F, 23A, 23B, and 23F. Only 5 of the 10 serotypes isolated were included in the conjugated heptavalent pneumococcal vaccine. Therefore, the other 5 serotypes (24 of 48 strains) should be considered in selecting antigens for the new vaccine.

KEY WORDS — acute otitis media, antimicrobial susceptibility, microbiology, *Streptococcus pneumoniae* conjugate vaccine, *Streptococcus pneumoniae* serotypes.

Antimicrobials are prescribed by physicians more often for acute otitis media (AOM) than for any other disease in the United States.[1,2] The high-frequency use of antimicrobials for empiric treatment of childhood ear infections has caused multi-antibiotic–resistant pathogens,[3] resulting in treatment failure for many patients.[3-5]

Streptococcus pneumoniae (15% to 35%), *Haemophilus influenzae* (nontypeable; 15% to 25%), and *Moraxella catarrhalis* (10% to 20%) are considered to be the major causes of AOM in the United States. These pathogens have developed resistance to the β-lactam drugs: penicillin, ampicillin, and the cephalosporins.[4,6-8] In some geographic areas, resistance to penicillin is found in around 40% of isolates of *S pneumoniae* from otitis media (OM) cases,[8,9] and resistance to amoxicillin has been found in around 90% of isolates of *M catarrhalis* and around 30% of isolates of *H influenzae* cases.[6] Resistance is developing, simultaneously, to antibiotics other than the β-lactams, eliminating all except 4 of the 16 drugs that were previously recommended in clinical practice for empirically treating AOM.[4-6] Only amoxicillin (high dosage, first line), amoxicillin-clavulanate for β-lactamase producers, axetil-cefuroxime, and ceftriaxone (intramuscular) are considered for use against resistant pathogens that cause AOM.[2,6,8]

Treatment failure in AOM can result in several important sequelae: recurrent OM requiring surgery in the form of myringotomy and tympanostomy tube placement,[4,6] hearing loss with cognitive and developmental implications,[10,11] and inappropriate use of antimicrobials that leads to the emergence and spread of resistant organisms.[9,12-14]

To follow the resistance and spread of *S pneumoniae*, which causes 7 million cases of OM each year, surveillance systems are in place in the United States.[7,15] After pneumococci are introduced into a susceptible population, resistant clones spread rapidly.[12,14-16] Because of the severity of the invasive diseases caused by this pathogen, attempts have been made to control *S pneumoniae* with a vaccine. Immunization against pneumococci, however, with a 23-valent polysaccharide vaccine has not been effective in very young children.[17] The newly tested conjugated heptavalent pneumococcal vaccine, PNCRM7, appears both relatively safe and effective.[18] It has been shown to generate a response against 7 antigenic pneumococcal serotypes in infants 2 years of age and younger. The vaccine contains only those serotypes that are most prevalent in the United States.[19,20] The conjugate vaccine for pneumococci has received market clearance and is now available (Wyeth-Lederle Vaccines and Pediatrics, West Henrietta, New York).

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(Use of trade names or commercial sources is for identification only and does not imply endorsement by the Public Health Service or by the US Department of Health and Human Services.)

Brazil has begun a comprehensive surveillance system to follow pneumococcal resistance,[21-23] and studies indicate a need for collecting microbiological evaluation data to establish guidelines for treating acute or recurrent OM. The present investigation was undertaken, first, to identify microorganisms and antimicrobial susceptibilities of pathogens from AOM in Brazilian children; next, to ascertain, by comparison, whether the isolates of *S pneumoniae* have the same serotypes as those included in the new conjugated heptavalent pneumococcal vaccine; and last, to determine whether additional and/or different serotypes should be included in the vaccine to ensure an immunogenic response against pneumococcal pathogens for the indigenous children in this study.

MATERIALS AND METHODS

Between 1990 and 1995, the study enrolled a total of 300 children with AOM. The children, 2 months to 5 years of age, were recruited in the emergency room at the Instituto da Criança Prof Pedro de Alcântara, Medical School, University of São Paulo.

Case Definition. Patients who, upon examination with a pneumatic otoscope, demonstrated the presence of middle ear effusion with a reddened and/or bulging tympanic membrane and had fever, otalgia, or irritability were considered to have AOM. Included were patients with the following signs or symptoms: anorexia, nausea, vomiting, vertigo, tinnitus, and nystagmus.

Requirements for Enrollment. Only children who had received no antibiotics for 2 months before the diagnosis of AOM were selected for enrollment. Patients were also excluded if the tympanic membrane was perforated or if the external ear canal showed apparent infection or trauma. Parents had to give consent and agree to medical follow-up for the child.

Preparation, Collection, and Culture of Specimens. The ear canal was irrigated for 2 minutes with 70% ethyl alcohol. No anesthesia was administered. The child was immobilized while myringotomy was performed, and middle ear fluid was aspirated into a sterile Juhn Tymp-Tap (Xomed, Jacksonville, Florida). One drop of the fluid was inoculated for culture in both brain-heart infusion containing 5% rabbit blood, and chocolate Isovitalex proteose base in agar. Two drops of fluid were inoculated into each of 3 broths: thioglycollate, brain-heart infusion, and Todd-Hewitt. The remaining fluid was placed in a cryovial with sterile rabbit blood, frozen, and transported in liquid nitrogen to the Respiratory Disease Laboratory, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia. The frozen fluid was cultured and microorganisms were confirmed at the CDC. The prevalent pathogens were serotyped and antibiotic susceptibility profiles were determined. Minimal inhibitory concentrations (MICs) for *H influenzae* were determined with *Haemophilus* test medium, according to protocol M7-A2 from the National Committee for Clinical Laboratory Standards (NCCLS).[24] Two panels were used to determine these MICs (Sensititer, Radiometer America, Westlake, Ohio; and Sceptor, Becton Dickinson, Towson, Maryland).

RESULTS

Risk Factors. The histories of the patients gathered at the time of diagnosis uncovered a variety of risk factors: 24% had siblings with recurrent OM episodes, 28% had a history of prior pneumonia, 38% attended day care centers, and 70% were exposed to passive smoking.

Simultaneous Symptoms. Of the 300 children who participated, 89% had nasal discharge, 95% had pharyngeal erythema, 46% had adenopathy, 60% had cough, and 3% had conjunctivitis.

Age. The children ranged in age from 2 to 60 months, with a mean age of 7.3 months. The mean number of OM recurrences by 5 years of age was 3.5.

Microorganisms Isolated. Microorganisms were isolated from the ear fluid of 192 patients (64%). The 5 most frequently isolated pathogens from the 300 patients were *S pneumoniae* (48 isolates; 16%), *H influenzae* (21 isolates; 7%), *M catarrhalis* (15 isolates; 5%), *Pseudomonas aeruginosa* (6 isolates; 2%), and coagulase-positive *Staphylococcus aureus* (3 isolates; 1%). In addition, multiple organisms that varied greatly were isolated from 99 of the children (33%). The isolates, listed in order of predominance, were *Pseudomonas* sp, *Staphylococcus* sp, *Streptococcus* sp, *Acinetobacter* sp, *Micrococcus* sp, *Candida albicans*, *Rhodococcus* sp, other *Candida* sp, *Alloiococcus otitidis*, *Stomatococcus* sp, *Moraxella osloensis*, CDC IIh, *Rhodotorula rubra*, *Flavobacterium* sp, *Xanthomonas maltophilia*, *Flavomonas* sp, and *Corynebacterium* A-4. No organisms were isolated from 108 patients (36%).

Serotypes. The *S pneumoniae* types, listed by prevalence, were 9 isolates each of serotypes 16 and 19F, 6 each of 19A and 6B, and 3 each of 9V, 11A, 18C, 23A, 23B, and 23F. The serotypes found in the ear fluid of these children and those found in the heptavalent vaccine[19,20] were compared. The serotypes in the vaccine are 4, 6B, 9V, 14, 18C, 19F, and 23F. Five of these 7 serotypes (24 of 48 strains) were isolated in this study: 9 of 19F, 6 of 6B, and 3 each of 9V, 18C, and 23F. However, 5 serotypes (24 of 48 strains) isolated in this study were not found in the vaccine: 9 of 16, 6 of 19A, and 3 each of 11A, 23A, and 23B.

Of the 21 *H influenzae* strains, 3 (14%) were type b and 18 were nontypeable. These nontypeable strains are not vaccine-preventable and, thus, remain as treatment problems for AOM. However, in the United States, type b *H influenzae* has practically been eliminated in patients who receive the HiB conjugated vaccine.[25,26]

Antimicrobials. The NCCLS[27] MIC cutoff value for interpreting intermediate or relative resistance of *S pneumoniae* to penicillin is between 0.12 and 1.0 µg/mL, and for high-level resistance, ≥2.0 µg/mL. Twenty-seven of the 48 *S pneumoniae* strains were considered intermediately or relatively resistant to penicillin; however, none were highly resistant. Twelve of the 48 *S pneumoniae* strains were highly resistant to trimethoprim-sulfamethoxazole (MIC of ≥4/78 µg/mL), and 9 were relatively resistant, with MIC values between 1/19 and 2/38 µg/mL. The *S pneumoniae* strains that demonstrated relative resistance to penicillin were also highly resistant to trimethoprim-sulfamethoxazole, and 3 of these were relatively resistant to cefaclor. No resistance was observed to ceftriaxone, cefotaxime, erythromycin, or chloramphenicol. The conventional method that uses oxacillin disks to detect resistance to penicillin was a reliable indicator for all of the resistant strains, even for those with MICs as low as 0.12 µg/mL.

Of the 21 isolates of *H influenzae*, 3 (14%) were β-lactamase–positive. Of the 3 isolates of type b *H influenzae*, 1 was β-lactamase–positive and resistant to ampicillin (MIC of ≥8 µg/mL), trimethoprim-sulfamethoxazole (MIC of ≥8/152 µg/mL), chloramphenicol (MIC of ≥16 µg/mL), and tetracycline (MIC of ≥8 µg/mL). Two type b *H influenzae* isolates were β-lactamase–negative; 1 was resistant to erythromycin (MIC of ≥8 µg/mL), and the other was intermediately resistant to trimethoprim-sulfamethoxazole. The presence of β-lactamase was detected in all β-lactam–resistant strains with the rapid cephalosporin reduction test. The 15 *M catarrhalis* strains were resistant to ampicillin (β-lactamase–positive); all were susceptible to amoxicillin–clavulanic acid and erythromycin.

DISCUSSION

In our study, the 3 most prevalent pathogens were *S pneumoniae* (16%), *H influenzae* (7%), and *M catarrhalis* (5%), and resistance to antibiotics was found in all 3 pathogens. Although these results are similar to findings from the United States and other countries,[2,3,8,9,20] this study shows 2 significant dissimilarities from the US situation. In our population, there was no high-level resistance of *S pneumoniae* to penicillin; instead, there was high-level resistance to trimethoprim-sulfamethoxazole. The higher rate of resistance to sulfa drugs in Brazil as compared to the United States is quite likely due to heavy usage of less-costly sulfa drugs. It is also not surprising that we observed a lack of high-level resistance to third-generation β-lactam drugs, because of their relative expense and low rate of use in Brazil. A need is indicated for application of modern epidemiological DNA tools, ribotypes, fingerprints, or probes to identify clonal resistant strains and to follow the spread of these strains to other patients.[12,16,28-30]

This study of acute cases, performed at the time of onset, cannot be compared with studies that have been conducted on recurring cases. The US studies have primarily involved patients who received multiple rounds of ineffective antibiotics.[5,6] The fluid for culture is obtained only after treatment is considered to be ineffective and a tympanostomy tube is placed to promote adequate middle ear pressure and ventilation.[6,31] Therefore, pathogens isolated at this chronic stage of infection may be either the original causative agents, which have developed antibiotic resistance after long exposure to ineffective drugs, or newly acquired resistant clones.

This study revealed a multiplicity of organisms in the middle ear fluid of AOM patients that are not considered to be causative agents for OM: *Pseudomonas* sp, *Staphylococcus* sp, *Streptococcus* sp, *Acinetobacter* sp, *Micrococcus* sp, *Candida albicans*, *Rhodococcus* sp, *Candida* sp, *Alloiococcus otitidis*, *Stomatococcus* sp, *Moraxella osloensis*, CDC IIh, *Rhodotorula rubra*, *Flavobacterium* sp, *Xanthomonas maltophilia*, *Flavomonas* sp, and *Corynebacterium* A-4. These organisms are considered to cause mostly self-resolving OM infections[2,5,6] and would therefore not commonly be given the status of pathogens in OM treatment plans. However, these organisms, if found in other sterile sites, would certainly be considered pathogenic. Even though their role in OM is not established, 99 of such organisms were isolated from ear fluid in 33% of the cases and must, at least, be considered as part of the effusive or "glue-ear" process that renders the ear vulnerable to other pathogens.

Brazil, at the time of this study, appears to be in the beginning phase of penicillin resistance by *S pneumoniae*.[21-23] Therefore, with judicious use of antibiotics, clinicians may be able to avoid the high-level resistance that is occurring in populations in other countries.[8,9,16]

To ensure an immunogenic response against pneumococcal pathogens for the indigenous children in this study, the new conjugated pneumococcal vaccine must contain the serotypes found in the endemic geographic area. Only 5 of the 10 serotypes isolated were included in the conjugated heptavalent vaccine. Therefore, the other 5 serotypes should be considered when selecting antigens for the new vaccine.[18,19] Because there are more than 80 pneumococcal serotypes, and because isolates from invasive diseases need to be compared with strains from AOM, this study indicates that continual surveillance is needed to determine the specific serotypes of an endemic area and subsequently to add these specific types to the vaccine. To ensure an immunogenic response against *S pneumoniae*, the data from this study suggest that vaccines should be customized to include the *S pneumoniae* serotypes indigenous to specific geographic locations. With this selective approach, there is the promise that the use of a conjugated vaccine can be as successful against pneumococci as it has been in eliminating type b *H influenzae*.[25,26,32]

ACKNOWLEDGMENTS — The author acknowledges the following group from the National Center for Infectious Disease for their technical assistance with the identification of pathogens, and for providing essential supplies for the study: Gail Bosley, Dannie Hollis, Maryam Daneshvar, June Brown, Bertha Hill, and Richard Facklam. The author acknowledges John Rex Astles, Gail Bosley, and Devery Howerton of the Public Health Practice Program Office for their assistance with editing and composing the manuscript.

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Ann Otol Rhinol Laryngol 110:2001

PARANASAL SINUS VENTILATION IN HEALTHY SUBJECTS AND IN PATIENTS WITH SINUS DISEASE EVALUATED WITH THE 133-XENON WASHOUT TECHNIQUE

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Ventilation of the paranasal sinuses is of great importance in sinus pathophysiology. Therefore, methods of measuring sinus ventilation are important for the evaluation of patients with sinus disease. In the present study, a 133-xenon washout technique was used to evaluate the ventilation of the paranasal sinuses in 34 healthy subjects and in 13 subjects with sinus disease (5 patients with nasal polyposis and 8 patients with chronic sinusitis). For this purpose, a 133-xenon–air mixture was insufflated in each nostril and the washout of the radioactive gas from the paranasal sinuses was monitored with a dynamic single-photon-emission computed tomography camera. The half-time (±SD) was found to be 18 ± 18 minutes for the maxillary sinus, 10 ± 8 minutes for the frontal sinus, and 18 ± 23 minutes for the posterior ethmoid and sphenoid sinuses in the healthy subjects. Repeated measurements in 18 of the healthy subjects indicated that the method had acceptable reproducibility according to a Bland-Altman plot. The 133-xenon washout was not influenced by insufflation pressure, nasal patency, or body position. The subjects with sinus disease exhibited half-times of 77 ± 101 minutes for the maxillary sinus, 91 ± 124 minutes for the frontal sinus, and 60 ± 60 minutes for the posterior ethmoid and sphenoid sinuses. For patients with nasal polyposis, the half-time was significantly longer than that in healthy subjects, while patients with chronic sinusitis did not differ from healthy subjects in this respect.

KEY WORDS — chronic sinusitis, nasal polyposis, ostial function, paranasal sinuses, single-photon-emission computed tomography, ventilation, 133-xenon.

INTRODUCTION

The proper functioning of the ostia of the paranasal sinuses is an important factor in sinus physiology and in the pathophysiology of sinusitis. Impairment of ostial function facilitates the development of sinusitis.[1] Both invasive and noninvasive methods have been used to study sinus ostial function.[2] Verstraelen[3] used a noninvasive 133-xenon washout technique to study sinus ventilation in normal subjects. In short, an air–133-xenon mixture is insufflated into the nose. The half-time ($T^1/2$) of xenon washout from the various paranasal sinuses is measured with a scintillation camera, and this is considered to be correlated to ostial function. We have previously used a similar noninvasive 133-xenon technique to study the variation in ventilation in the various sinuses of healthy subjects.[4]

In order to validate the 133-xenon washout technique properly, it is of great importance to study its reproducibility, which, to the best of our knowledge, has not been studied in detail. The aim of the present investigation was to obtain reference values for sinus ventilation and to study the reproducibility of repeated measurements made with the 133-xenon washout technique in a representative series of healthy subjects. Furthermore, the influence of posture, nasal patency, and insufflation pressure was investigated.

A new concept in the surgical treatment of chronic sinusitis has recently been developed, ie, functional endoscopic sinus surgery (FESS). This technique is intended to restore normal sinus ventilation and drainage. Thus, it is of considerable interest to develop methods for measuring ventilation of the paranasal sinuses both before and after operation, for objective validation of FESS results. Therefore, our second aim was to study the sinus ventilation in patients with sinus disease and to compare the results with those from healthy subjects.

SUBJECTS AND METHODS

Subjects. The control group comprised 34 healthy

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This study was supported by grants from the Stig and Ragna Gorthon Foundation for Medical Research, The Thelma Zoéga Foundation for Medical Research, and the Swedish Medical Research Council (project 7940).

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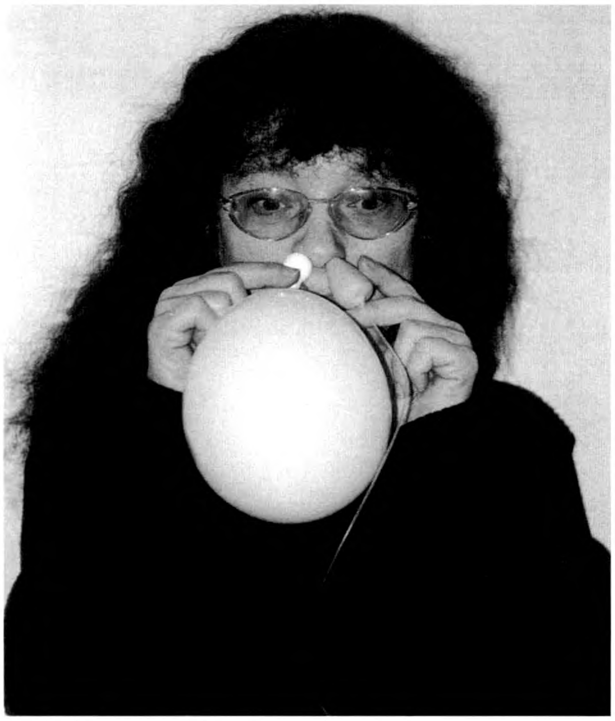

Fig 1. Technique for insufflation of 133-xenon into paranasal sinuses. Subject is seen inflating balloon connected to right nostril after injection of air–133-xenon mixture into left nostril via olive-tipped catheter.

subjects (24 men and 10 women; mean age, 37 years; range, 18 to 60 years) with no record of upper respiratory tract infection within the preceding 4-week period. Sinus radiographs, obtained for 27 subjects, showed normal results. In the remaining 7 subjects, ultrasonography of the sinuses showed normal results.

The patient group consisted of 13 subjects (8 men and 5 women; mean age, 33 years; range, 24 to 50 years) with sinus disease. A computed tomography (CT) scan of the paranasal sinuses was performed on all the patients. Eight of the patients had chronic sinusitis, and 5 had nasal polyposis. Two of the patients had had previous sinus surgery, but now presented with recurrent disease. The disease shown on the CT scan was graded in terms of mucosal swelling as none, slight, moderate, or pronounced (almost occluding the sinus).

The study design was approved by the Ethics Committee, Faculty of Medicine, Lund University, and the Radiation Safety Committee of Helsingborg Hospital.

133-Xenon Insufflation and Washout From Paranasal Sinuses. The 133-xenon washout was measured in the 34 healthy subjects. Two measurements were performed in 18 of the subjects with an interval of about 24 hours. The technique used for insufflation of 133-xenon into the sinuses was a modified version of a method described previously.[4] In the majority of the subjects, the nose was rinsed with physiological saline solution before insufflation. An increase in pressure in the nasal cavity during insufflation facilitates the passage of 133-xenon gas into the sinuses. Therefore, a rubber balloon, developed for the treatment of serous otitis media by increasing intranasal pressure (Otovent, Viborg, Denmark), was connected to the nostril contralateral to the nostril used for insufflation. To accustom the subjects to the insufflation procedure, we instructed them to inflate the balloon twice on both sides before xenon insufflation. For insufflation, about 200 MBq of 133-xenon gas was mixed with air in a 60-mL syringe. Fifteen milliliters of this mixture was insufflated during 10 seconds in 1 nostril through an olive-tipped catheter while the subject held his or her breath. Immediately after insufflation, with the catheter still connected, the subject inflated the balloon and kept it inflated for 15 to 20 seconds before starting normal mouth breathing (Fig 1). The procedure was then repeated in the contralateral nasal cavity. The insufflation procedure was performed once more in each nostril. After the insufflation procedure, the nose was clamped until the measurements were initiated. The time (±SD) between starting insufflation and starting the measurements, measured in 10 subjects, was 6 ± 1.8 minutes (range, 3 to 11 minutes).

The subjects were instructed to breathe normally through the nose during measurements. The measurements were carried out with the subjects in the supine position with a scintillation camera for dynamic single-photon-emission CT (SPECT; Toshiba GCA-901 A/ECT-S4, Tokyo, Japan). The SPECT camera head executed an anterior rotation of 180° in 1 minute, with 12° sampling angles, and the rotation was repeated automatically every minute for 30 minutes. Three-dimensional regions of interest (ROIs) were selected for the frontal, maxillary, posterior ethmoid, and sphenoid sinuses from the activity images thus obtained. The number of counts per minute for the ROIs representing the sinuses was calculated and monitored for 30 minutes. These values were then plotted to give 133-xenon washout curves (Fig 2). Exponential curve fitting was applied, by means of the software supplied with the Toshiba camera. The $T^1/_2$ of the fitted curve was adopted as the $T^1/_2$ of the 133-xenon washout.

The initial activity for each sinus was estimated in 10 healthy subjects. These subjects were examined twice in the supine position and once in a sitting position. From the ROIs, the number of counts during the first minute was calculated for each sinus. Furthermore, these numbers of counts were corrected for the sensitivity of the camera (counts per

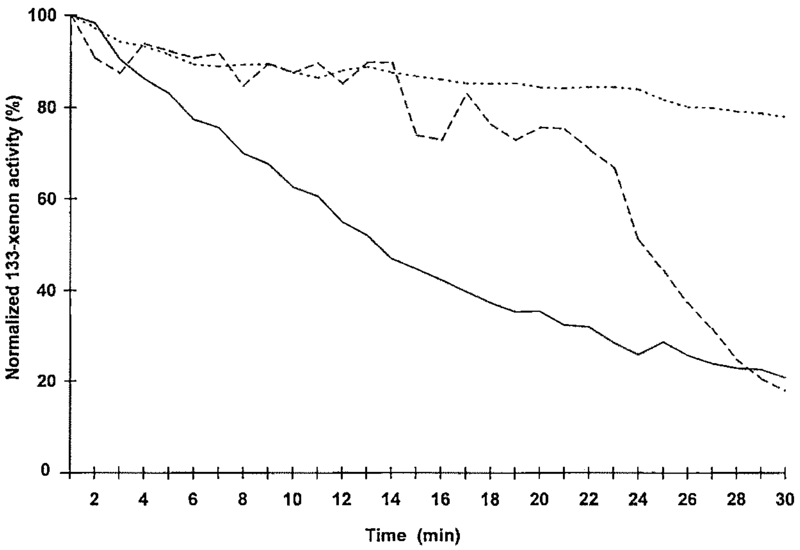

Fig 2. Examples of 133-xenon washout curves. Typical monoexponential 133-xenon washout curve (unbroken line) and more seldom observed biphasic washout curve (dashed line) from maxillary sinuses in 2 healthy subjects. Dotted line represents washout curve from maxillary sinus in patient with chronic sinusitis (patient 12 in Table 2).

minute per megabecquerel) and for tissue attenuation due to the assumed depths of the different sinuses. From the corrected counts, the initial activity could be calculated in megabecquerels and expressed as a percentage of the total amount of insufflated radioactivity.

Nasal Patency and Insufflation Pressure. Anterior rhinomanometry was performed with a rhinomanometer (RM-2100, SRE, Copenhagen, Denmark) in 10 healthy subjects before and 15 minutes after rinsing the nose with physiological saline solution. Afterward, the insufflation pressure was measured during inflation of the balloon before insufflation of the air-xenon mixture. The measurements were performed in the nasal cavity contralateral to the balloon with a catheter connected to a pressure recorder (Synectics Medical, Stockholm, Sweden). The procedure was carried out twice for each nasal cavity. The peak insufflation pressure was calculated as the mean of the 2 peak values for each nostril.

Influence of Posture. Nine of the healthy subjects were examined in both supine and sitting positions. For the examination in the sitting position, a scintillation camera without the SPECT capability (Siemens LFOV, Searle, Uithoorn, the Netherlands), connected to a computer (ADAC Laboratories, Milpitas, California), was used. These planar images did not allow adequate analysis of the posterior ethmoid and sphenoid sinuses.

Statistics. All results are expressed as means ± SD, unless otherwise stated. The mean $T^1/2$ values for healthy subjects were calculated after the exclusion of extreme outliers according to Tukey [third quartile + 3 × (third quartile – first quartile)]. For intraindividual comparisons between the $T^1/2$ values for the different paranasal sinuses in healthy subjects, the Wilcoxon signed rank test was used. For interindividual comparisons between the $T^1/2$ values of paranasal sinuses in the healthy subjects and the patients, the Mann-Whitney U-test was performed with SPSS software (SPSS, Chicago, Illinois). Linear regression analysis and Spearman rank correlation analysis were performed with Epistat software (Epistat, Richardson, Texas). The Kruskal-Wallis test was used to compare the grade of disease determined from the CT scans with the $T^1/2$ for the same sinus (SPSS). The differences in proportions were analyzed with Fisher's exact test. We considered p values of less than .05 to be statistically significant. Two-tailed tests were used throughout.

To validate the agreement between 2 xenon washout $T^1/2$ values (determined with a 24-hour interval) for a given subject, we plotted the difference in the values against the mean of the same 2 values to obtain the optimal approximation of the true value of sinus ventilation. This statistical method has been described by Bland and Altman.[5] In a Bland-Altman plot, the equivalent of the ideal correlation coefficient (r = 1) is a difference between repeated measurements of 0. The agreement between measurements is considered good if the interval between the standard deviations of the differences is small.

RESULTS

133-Xenon Washout From Paranasal Sinuses in Healthy Subjects. The individual 133-xenon washout $T^1/2$ values are given in Table 1 for the healthy subjects. Mean values were calculated for right maxillary, left maxillary, frontal and posterior ethmoid, and sphenoid sinuses after the exclusion of 6 far outliers. The mean $T^1/2$ for the right maxillary sinus was found to be 16 ± 15 minutes (n = 33), and that for the left maxillary sinus was 20 ± 20 minutes (n = 33). The difference between the values for the right and

TABLE 1. HALF-TIME IN MINUTES OF 133-XENON WASHOUT FOR PARANASAL SINUSES IN HEALTHY SUBJECTS

	Sinus			
Subject No.	*Right Maxillary*	*Left Maxillary*	*Frontal*	*Posterior Ethmoid and Sphenoid*
1	15	17	110*	15
2	98*	35	31	28
3	1	37	18	56
4	4	8	13	3
5	6	NA	8	NA
6	19	4	35	48
7	26	8	14	4
8	9	32	1	NA
9	6	6	2	28
10	1	3	NA	545*
11	15	40	4	NA
12	19	11	6	NA
13	3	2	6	2
14	39	19	8	12
15	12	2 (88)†	9	2
16	19	5	4	NA
17	42	10	7	9
18	11	23	7	9
19	28	22	25	230*
20	2	6	7	3
21	18	5	39*	101
22	31	32	6	5
23	1	9	10	5
24	2	13	5	10
25	23	38	13	31
26	1	47	4	3
27	2	99	6	22
28	6	4	6	3
29	10	27	3	NA
30	23	26	5	2
31	72	1	6	124*
32	32	33	10	23
33	12	38	2	9
34	7	3	13	6
Median	12	13	7	10
Q3 - Q1	18	27	8	25
Mean	16	20	10	18
SD	15	20	8	23

NA — no activity in that sinus; Q3 – Q1 — third quartile minus first quartile.

*Far outlier (according to Tukey).

†Half-time for first 2 minutes. Number in parentheses denotes half-time for remaining period of measurement.

left maxillary sinuses was not significant according to the Wilcoxon signed rank test, and the common mean for the maxillary sinuses was calculated, giving 18 ± 18 minutes (n = 66). The T^{1}/2 values for the posterior ethmoid and sphenoid sinuses measured together were 18 ± 23 minutes (n = 25). The mean T^{1}/2 for the frontal sinus was 10 ± 8 minutes (n = 31), and this was found to be significantly lower than the value for the maxillary sinus.

The differences between the first and second measurements carried out on 18 of the healthy subjects were plotted against the mean of the first and second measurements, according to Bland and Altman.[5] The mean difference for the maxillary sinus was 6 ± 20 minutes (n = 32; Fig 3), that for the frontal sinus was 2 ± 9 minutes (n = 17; Fig 4), and that for the posterior ethmoid and sphenoid sinuses was 7 ± 25 minutes (n = 11; not shown). For these 18 subjects, monoexponential washout curves were obtained for all measurements but 2, in which biphasic washout curves were observed for the maxillary sinuses.

The initial activity for the first minute of the measurements was 5,004 ± 3,512 counts per minute (n = 28) in the right maxillary sinus, 5,118 ± 3,775 counts per minute (n = 28) in the left maxillary sinus, 2,879 ± 1,853 counts per minute (n = 26) in the frontal sinus, and 1,515 ± 1,060 counts per minute (n = 14) in the posterior ethmoid and sphenoid sinuses. The initial activity expressed in percent of the insufflated activity was 1.0% ± 0.8% in the right maxillary sinus, 0.9% ± 0.7% in the left maxillary sinus, 0.6% ± 0.4% in the frontal sinus, and 0.7% ± 1.5% in the posterior ethmoid and sphenoid sinuses. Spearman rank correlation analysis suggested a positive correlation between initial activity and T^{1}/2 values for the second maxillary sinus measurements (r = .55; p = .05) and for the first frontal sinus measurements (r = .80; p < .01). No correlation was found in any other series of measurements.

Influence of Nasal Patency and Insufflation Pressure. After the nose was rinsed with physiological saline solution, the inspiratory nasal airway resistance was studied in 10 healthy subjects and was found to be 0.8 ± 1.3 Pa • s/mL for the right nasal cavity and 0.6 ± 0.6 Pa • s/mL for the left nasal cavity. The "total nasal resistance" was 0.3 ± 0.2 Pa • s/mL. Spearman rank correlation analysis showed no significant relationship between nasal airway resistance and the 133-xenon washout T^{1}/2 for the maxillary, frontal, or posterior ethmoid and sphenoid sinuses.

The peak insufflation pressure was measured in 10 healthy subjects and was found to be 65 ± 17 hPa for the right nasal cavity and 62 ± 12 hPa for the left nasal cavity. Spearman rank correlation analysis showed no significant relationship between the insufflation pressure and the 133-xenon washout from the paranasal sinuses, nor was any correlation found to exist between the insufflation pressure and the ini-

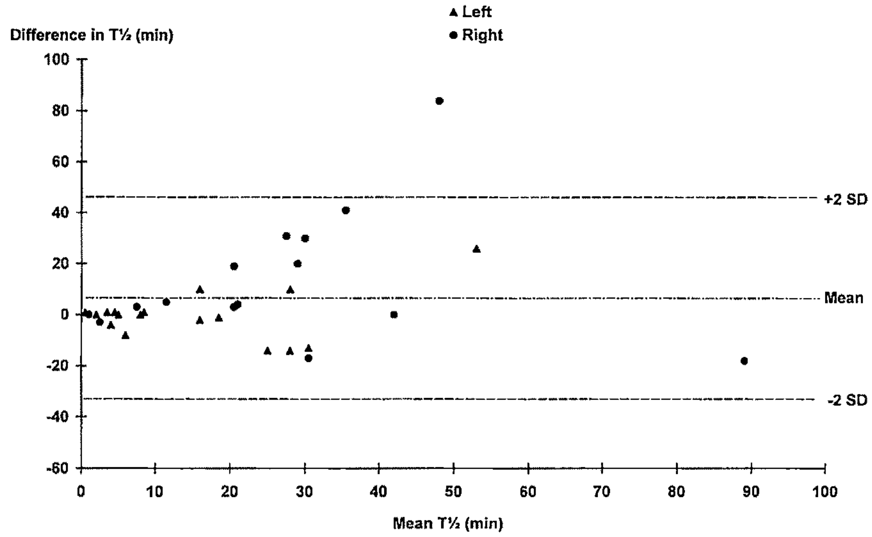

Fig 3. Bland-Altman plot of mean of half-time ($T^1/2$; abscissa) and difference in $T^1/2$ (ordinate) for 32 maxillary sinuses in repeated measurements of 133-xenon washout in 18 healthy subjects. No activity was observed in 2 measurements, and there were also 2 far outliers.

tial activity in the sinuses.

Influence of Posture. The mean 133-xenon washout $T^1/2$ for healthy subjects in the sitting position was 13 ± 10 minutes (n = 18) for the maxillary sinus and 6 ± 3 minutes (n = 8) for the frontal sinus. A Bland-Altman plot showed mean differences between sitting $T^1/2$ and the supine $T^1/2$ for the maxillary sinuses of 4 ± 25 minutes (n = 18) and 1.6 ± 0.9 minute (n = 8) for the frontal sinus (not illustrated). There was no statistically significant difference between measurements in the supine and sitting positions.

133-Xenon Washout From Paranasal Sinuses in Patients. The individual 133-xenon washout $T^1/2$ values for the 13 subjects with sinus disease are listed in Table 2. The mean values for all patients were for the maxillary sinus, 77 ± 101 minutes (n = 22); for the frontal sinus, 91 ± 124 minutes (n = 10); and for posterior ethmoid and sphenoid sinuses, 60 ± 60 minutes (n = 8). These values do not significantly differ from the values for healthy subjects. Similarly, there was no significant difference between the values for the paranasal sinuses in the patients with chronic sinusitis and the healthy subjects. However, the difference between the washout $T^1/2$ values for the paranasal sinuses in the patients with nasal polyposis and the healthy subjects was significant, except for the left maxillary sinus (Tables 2 and 3).

At the beginning of the measurements, no activity was observed in 4 maxillary sinuses, 3 frontal sinuses, or 5 posterior ethmoid and sphenoid sinuses in the patient group (Table 2). The number of measurements that showed no activity was significantly higher in the maxillary sinus in the patients than in the healthy subjects, but no such significant difference was found for the frontal sinus or the posterior ethmoid and sphenoid sinuses.

Assuming that a correlation might exist between the $T^1/2$ for 133-xenon washout and the degree of mucosal swelling observed in the CT scan, we analyzed these variables using the Kruskal-Wallis test

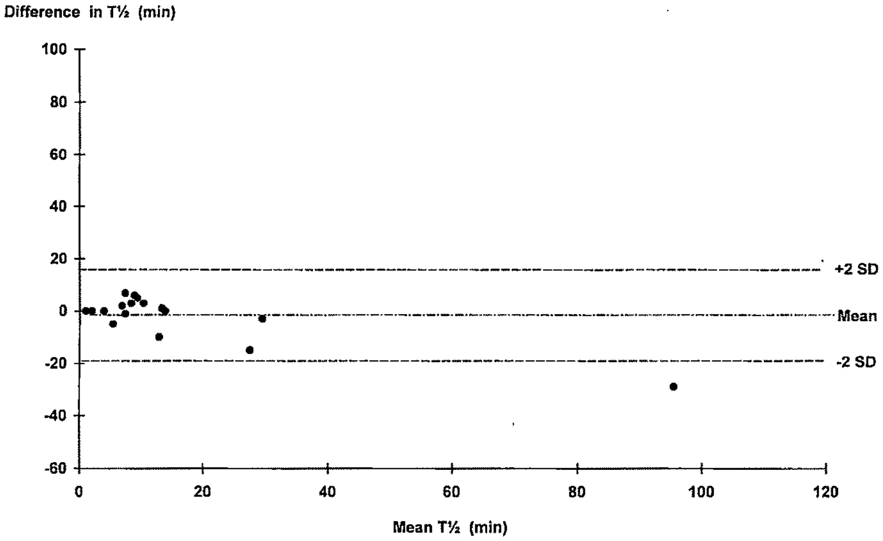

Fig 4. Bland-Altman plot of mean of $T^1/2$ (abscissa) and difference in $T^1/2$ (ordinate) for frontal sinus in repeated measurements of 133-xenon washout in 17 healthy subjects. No activity could be measured in 1 subject because of lack of frontal sinus.

TABLE 2. CLINICAL DATA AND RESULTS OF 133-XENON WASHOUT IN 13 PATIENTS WITH SINUS DISEASE

			*Computed Tomography by Sinus**					*Half-Time by Sinus*				
Patient No.	*Age (y)*	*Sex*	*Right Maxillary*	*Left Maxillary*	*Frontal*	*Posterior Ethmoid and Sphenoid*	*Previous Surgery†*	*Right Maxillary*	*Left Maxillary*	*Frontal*	*Posterior Ethmoid and Sphenoid*	*Diagnosis*
1	29	F	+	+	–	++	No	23	3	NA	4	CS
2	33	M	–	–	–	–	No	6	7	10	NA	CS
3	36	F	–	–	–	–	No	5	2	10	NA	CS
4	24	M	+++	+++	–	+	Yes	NA	NA	91	107	P
5	30	F	++	++	+++	–	Yes	29	7	100	NA	CS
6	23	M	++	++	+	+++	No	NA	NA	NA	89	CS
7	30	M	++	++	+	++	No	202	94	325	NA	P
8	35	F	–	++	–	–	No	2	7	4	5	CS
9	31	M	+	–	–	++	No	86	278	40	152	P
10	37	M	+	+	–	+++	No	314	23	307	109	P
11	33	M	+	++	++	+	No	185	17	NA	NA	P
12	33	M	–	+	–	–	No	11	215	23	6	CS
13	50	F	–	+	–	–	No	6	173	2	11	CS
Median	33							23	17	32	50	
Q3 – Q1	5							30	127	88	102	
Mean ± SD	33 ± 7							79 ± 107	76 ± 101	91 ± 124	60 ± 60	
Mean ± SD (CS)								12 ± 10	59 ± 93	25 ± 38	23 ± 37	
Mean ± SD (P)								197 ± 93	103 ± 122	191 ± 146	123 ± 25	

NA — no activity, CS — chronic sinusitis, P — nasal polyposis.

*Mucosal swelling: none (–), slight (+), moderate (++), or pronounced (+++).

†Any type of previous nasal or sinus surgery.

for nonparametric comparison of groups. This analysis indicated that there were significant differences between T$^1/_2$ values for sinuses showing normal mucosa on the CT scan and sinuses in which the CT scan showed abnormal mucosa. However, we found no significant correlation between an increase in T$^1/_2$ and mucosal swelling (Fig 5).

DISCUSSION

In previous investigations, a conventional, planar scintillation camera was used to study 133-xenon washout from paranasal sinuses.[3,6,7] We used a tomographic scintillation camera (SPECT) in a previous study,[4] as well as in the present study. The reason was that SPECT gives a 3-dimensional view of the sinuses and therefore better resolution for selecting ROIs for the different sinuses. This seems to us particularly important in studying the posterior ethmoid and sphenoid sinuses. In our previous study,[4] the insufflation procedure included Valsalva's maneuver, which was difficult to standardize in different subjects. In the present investigation, the procedure was modified by use of a balloon that made it possible for the subjects to attain a more uniform increase in intranasal pressure. The insufflation technique is of major importance in achieving sufficient initial activity in the paranasal sinuses for the measurement of 133-xenon washout. In some series of measurements in the maxillary and frontal sinuses, a positive correlation was found between the initial activity and the T$^1/_2$ of 133-xenon washout. Hence, a short T$^1/_2$, possibly due to a wide ostium, would give a low initial activity, because some 133-xenon would have disappeared during the delay of 5 to 10 minutes between insufflation and the beginning of the measurements. On the other hand, the insufflation pressure was not correlated to the T$^1/_2$, so the interindividual variation in the intranasal pressure with the balloon technique does not affect the washout times. Wash-

TABLE 3. COMPARISON OF 133-XENON WASHOUT FOR PARANASAL SINUSES OF HEALTHY SUBJECTS AND PATIENTS BY MANN-WHITNEY U-TEST

	Healthy Subjects vs Patients With Chronic Sinusitis (p)	*Healthy Subjects vs Patients With Polyposis (p)*
Right maxillary sinus	.602 (34/7)	.001* (34/4)
Left maxillary sinus	.708 (33/7)	.070 (33/4)
Frontal sinus	.625 (33/6)	.002* (33/4)
Posterior ethmoid and sphenoid sinuses	.840 (28/5)	.023* (28/3)

Numbers within parentheses are numbers of healthy subjects/patients.

*Significant ($p < .05$).

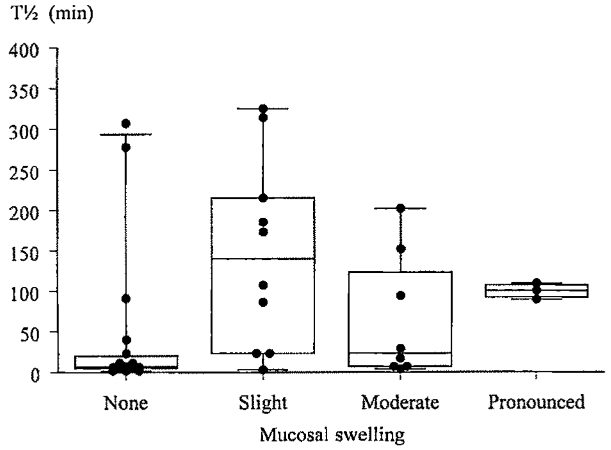

Fig 5. Box-and-whisker plot of T½ of 133-xenon washout for paranasal sinuses for 4 degrees of mucosal swelling (none, slight, moderate, and pronounced) seen on computed tomography scans of patients with chronic sinusitis and nasal polyposis. Box indicates lower and upper quartiles, and central line is median. "Whiskers" indicate 2.5% and 97.5% values. Dots represent individual values.

out times were also unrelated to nasal patency, as determined by anterior rhinomanometry. Thus, we found no evidence to support the view that moderate nasal blockage, such as that caused in healthy subjects by the nasal cycle, influences sinus ventilation. However, this does not exclude the possibility that passage through the ostia, and subsequently the 133-xenon washout, is influenced by nasal blockage caused by common colds or nasal polyps.

Bland-Altman plots[5] were used to study the intraindividual reproducibility of measurements in 18 healthy subjects. This graphic representation of repeated measurements is considered superior to routine correlation analysis, as it not only takes into account the variation between measurements, but it also reveals any tendency for the variation to depend on the actual mean of the observations. In the present study, this analysis indicated acceptable reproducibility for the 133-xenon washout method. However, we found the difference between 2 measurements of T½ to increase with the mean value of T½ in the Bland-Altman plots. A tentative explanation might be that a narrow ostium is more sensitive to mucosal swelling or occluding debris, resulting in a greater variation in sinus ventilation. On the other hand, a wide ostium might lead to a rapid washout of the insufflated activity. This latter hypothesis gains some support from the fact that in 2 healthy subjects, all activity had disappeared before 1 of the measurements of their maxillary sinuses, and the T½ was very short in the other measurement. Variations in T½ within 1 of the 30-minute measurements occurred in 2 subjects, suggesting that even in individuals without sinus problems, there are periods of variable sinus ventilation, probably reflecting normal physiological variation of ostial patency or possibly nasal breathing. The finding of biphasic washout curves in the maxillary sinus (Fig 2) in some healthy subjects could explain the occurrence of outliers and might complicate the interpretation of results from patients with sinus disease. It should be noted, however, that only 6 of 136 T½ measurements (Table 1) were considered to be outliers, with values of T½ to the far right of the main body of the data, and representative T½ values were obtained in 94% of the measurements. Thus, the risk of misinterpretation appears to be small.

In previous studies of sinus ventilation,[3,4,6-9] 133-xenon washout times were estimated for different sinuses. The values for the maxillary and frontal sinuses in the present study are similar to those found in previous studies. The results of our study indicate that the xenon washout times in healthy maxillary and frontal sinuses are about 18 ± 18 minutes ($n = 66$) and 10 ± 8 minutes ($n = 31$), respectively. These results suggest that a washout time longer than 54 minutes (mean + 2 SD) for the maxillary sinus and 26 minutes for the frontal sinus may reflect weak ostial function.

The finding of shorter washout times in frontal sinuses, compared with those for the other paranasal sinuses, in healthy subjects does not necessarily mean that ventilation is faster. It could also be an effect of the lower volume of the frontal sinuses, leading to a shorter T½. We have found in model experiments that the T½ increases with the sinus volume (Paulsson et al, unpublished data). For the posterior ethmoid and sphenoid sinuses, the T½ was 18 ± 23 minutes ($n = 25$). The ostial function for the posterior ethmoid and sphenoid sinuses is more complex than that for the other sinuses, as is probably reflected by the relatively high number of measurements showing no activity in the region (Table 1).

A tendency for washout to be more rapid in the sitting position than in the supine position was reported by Verstraelen.[3] This finding was not confirmed in the present study, since we found good agreement between sitting and supine measurements. Thus, body position does not appear to affect sinus ventilation in healthy subjects.

Knowledge regarding sinus ventilation in patients with sinus disease is limited. In the present study, a systematic comparison of sinus ventilation in patients with chronic sinusitis and nasal polyposis has been performed. We used the mean washout times for paranasal sinuses in healthy subjects as reference values for evaluation of the ventilation in patients (Table 3).

Interestingly, the $T^1/2$ for paranasal sinuses in patients with nasal polyposis was significantly longer than that in the healthy subjects, but not longer than that in patients with chronic sinusitis. That the patients with chronic sinusitis in our study were examined while in a fairly healthy condition might explain the normal sinus ventilation and the low grade of disease in some of the patients according to the CT scan (Table 2). A weak correlation was found between a prolonged $T^1/2$ and the observation of disease on the CT scan. Thus, the finding of mucosal swelling on CT may indicate weak ostial function. On the other hand, mucosal swelling reduces the volume of the sinus, and thus should lead to a short washout time. This tendency might explain why there was no correlation between an increase in $T^1/2$ and the grade of mucosal swelling as seen on CT (Fig 5).

In conclusion, the 133-xenon washout technique gives reproducible results in healthy subjects that are unaffected by nasal patency and body position and interindividual differences in insufflation technique. Moreover, the findings in the present study indicate that the 133-xenon washout technique is suitable for preoperative and postoperative evaluation of FESS procedures. Whether the clinical outcome of FESS procedures is also related to 133-xenon washout time is not known, and warrants further investigation.

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Ann Otol Rhinol Laryngol 110:2001

CONCENTRATIONS OF SERUM IMMUNOGLOBULINS AND ANTIBODIES TO PNEUMOCOCCAL CAPSULAR POLYSACCHARIDES IN PATIENTS WITH RECURRENT OR CHRONIC SINUSITIS

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A study was carried out to search for underlying immunoglobulin deficiencies in 25 patients with recurrent or chronic sinusitis. The mean duration of the patient histories of recurrent or chronic sinusitis was 7.2 years. Concentrations of serum immunoglobulins and specific pneumococcal antibodies were measured in the patients and in 25 age- and sex-matched control individuals. The mean serum IgA concentration (1.6 g/L) was lower in the patients than in the control individuals (2.1 g/L, $p = .024$). On the other hand, the mean serum concentration of IgG antibodies to pneumococcal type 14 polysaccharide was higher in the patients (2.54 μg/mL) than in the control individuals (0.92 μg/mL, $p = .008$). However, elevated concentrations of IgG antibodies to pneumococcal type 14 polysaccharide were detected mainly in patients with the highest serum IgA concentrations. The results suggest that in a subpopulation of patients with a long-lasting history of sinusitis, a low serum IgA concentration may be associated with a susceptibility to sinusitis.

KEY WORDS — antibodies to *Haemophilus influenzae* type b polysaccharide, antibodies to pneumococcal capsular polysaccharides, IgA, IgG subclass, immune deficiency, immunoglobulin allotype, immunoglobulin, sex distribution, sinusitis.

INTRODUCTION

Patients who have severe defects in antibody-mediated immune functions, such as X-linked agammaglobulinemia, common variable immunodeficiency, and immunodeficiency with elevated IgM, suffer from recurrent pyogenic infections, such as pneumonia, otitis media, and sinusitis.[1,2] The above-mentioned immune deficiency states are rare, but their association with sinusitis suggests that in patients who present with recurrent sinusitis, antibody-mediated immune responses may be important in the defense against the invading microorganisms. To evaluate antibody-mediated immune functions in patients with recurrent or chronic sinusitis, we measured serum immunoglobulin concentrations, IgG3 constant region gene allele G3m(b) and G3m(g) frequencies, and concentrations of serum antibodies to 4 different pneumococcal capsular polysaccharides and to *Haemophilus influenzae* capsular polysaccharide type b.

METHODS

Patients. The study patients were selected from 198 adult patients who were admitted from 1995 to 1997 to Jorvi Hospital, Espoo, Finland, for operative treatment because of recurrent or chronic sinusitis and who were evaluated and operated on by one of the authors (O.T.). Twelve patients with recurrent sinusitis and 13 patients with chronic sinusitis were included in the study. The patients represented a subpopulation of patients having the most severe spectrum of sinusitis symptoms of the 198 patients evaluated. The inclusion criteria for patients with recurrent sinusitis were more than 4 episodes of acute sinusitis during the past 12 months or more than 2 episodes of acute sinusitis a year during the past 5 years. Between the acute sinusitis episodes, the patients were relatively free of sinonasal symptoms. A diagnosis of acute sinusitis was made when effusion was detected in the maxillary sinuses by maxillary sinus punctures or sinus radiographs. Patients with chronic sinusitis had continuous symptoms of sinus inflammation manifesting as continuous stuffiness of the nose with nasal discharge that had lasted for more than 2 years with periodic exacerbations of purulent sinusitis. Patients who had received peroral cortico-

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steroid therapy within the past 12 months were excluded from the study.

The mean age of the patients was 45.5 years (range, 19 to 64 years). Eighteen of the study patients were female, and 7 were male. The mean preoperative duration of the history of symptoms related to recurrent or chronic sinusitis was 7.2 years (range, 1 to 22 years). Nine of the patients had asthma. Four patients had specific serum IgE antibodies to pollen or animal dust antigens. The mean Lund-Kennedy score[3] describing the opacification of the nasal sinuses as evaluated by computed tomography was 14.2 (range, 1 to 22). Computed tomographic imaging for the radiologic scoring was not performed during acute exacerbations of sinonasal disease. Nasal polyposis was present in 15 patients.

The patients were all operated on with the Messerklinger endoscopic sinus surgery technique. Thirteen study patients had 1 sinus operation, and the rest had 2 to 5 operations. Two of the study patients were operated on endoscopically by a modified Lothrop procedure to drain the frontal sinuses.

To evaluate the sex distribution of the patients undergoing endoscopic sinus surgery, we reviewed the ICD-10 (International Classification of Diseases, 10th revision[4]) surgical procedure codes of 435 patients operated on at Jorvi Hospital from January 1997 to September 1999.

The study was approved by the Review Board of Ethical Matters in the Department of Otorhinolaryngology, Helsinki University Central Hospital.

Serum Immunoglobulin Concentrations. Serum samples were taken during a follow-up visit 6 months after the surgery. Although the severity of the symptoms as experienced by the patients decreased after the operations, the majority of the study patients continued to have sinonasal symptoms after surgery. However, during the 6-month follow-up visit, the patients were relatively free of symptoms. Serum samples were obtained from age- and sex-matched control individuals, ie, healthy volunteers who did not have a history of sinusitis.

Concentrations of IgM, IgG1, IgG2, IgG4, and IgA were determined by nephelometry on a Behring BN100 nephelometer with reagents from Dade Behring, Marburg, Germany. Concentrations of IgG3 were determined with the same instrument and the latex-enhanced nephelometry kit LK008.G from The Binding Site, Birmingham, England. Total serum IgE concentrations were measured by the Pharmacia CAP-IgE method (Pharmacia, Uppsala, Sweden).

IgG3 Gm Allotypes. The IgG3 Gm allotypes were determined by enzyme immunoassay as described previously.[5]

Antibodies to Pneumococcal Capsular and Haemophilus influenzae *Type b Polysaccharides.* Determinations of antibody concentrations were performed at the National Public Health Institute, Helsinki. An enzyme-linked immunosorbent assay was used to measure serum IgG anti-pneumococcal capsular polysaccharide antibody concentrations.[6] Microtiter plates were coated with 10 μg/mL of 6B and 19F and 5 μg/mL of 14 and 23F pneumococcal polysaccharides (American Type Culture Collection, Rockville, Maryland). The anti-pneumococcal polysaccharide concentrations were calculated by comparing the titers of the samples to the titer of the reference serum 89-SF1 (received from the US Food and Drug Administration, Bethesda, Maryland). The results are given in micrograms per milliliter calculated on the basis of the officially assigned IgG values of the 89-SF1 reference serum. The lower detection limits were 0.11, 0.14, 0.17, and 0.09 μg/mL for types 6B, 14, 19F, and 23F, respectively. Total antibody to *H influenzae* type b polysaccharide was assayed by enzyme-linked immunosorbent assay.[7] The lower detection limit for total antibodies to *H influenzae* type b polysaccharide was 0.06 μg/mL.

Statistical Methods. The differences in serum immunoglobulin and antibody concentrations between the patients and the age- and sex-matched controls were tested with Student's *t*-test for paired samples. The correlation between serum IgA concentrations and serum IgG antibodies to pneumococcal type 14 polysaccharide was estimated by the Pearson product moment correlation coefficient. The nonequivalence of the sexes among patients with sinusitis was tested with the χ^2 test.

RESULTS

Serum Immunoglobulins. The serum IgM, IgG1, IgG2, IgG3, IgG4, IgA, and IgE concentrations were determined in 25 patients with recurrent sinusitis and in 25 age- and sex-matched healthy control individuals (Fig 1 and Table 1). There was no statistically significant difference in serum IgM, IgG subclass, or IgE concentrations between the patients with sinusitis and the healthy control individuals. The mean serum IgA concentration was slightly lower in the patients with sinusitis (mean, 1.6 g/L; range, 0.53 to 2.8 g/L) than in the healthy control individuals (mean, 2.1 g/L; range, 0.50 to 4.9 g/L; p = .024). There was no statistically significant difference in the IgG3 Gm allotype b and g frequencies between the patients with sinusitis and the healthy individuals (Table 2[8]).

Concentrations of Antibodies to Pneumococcal

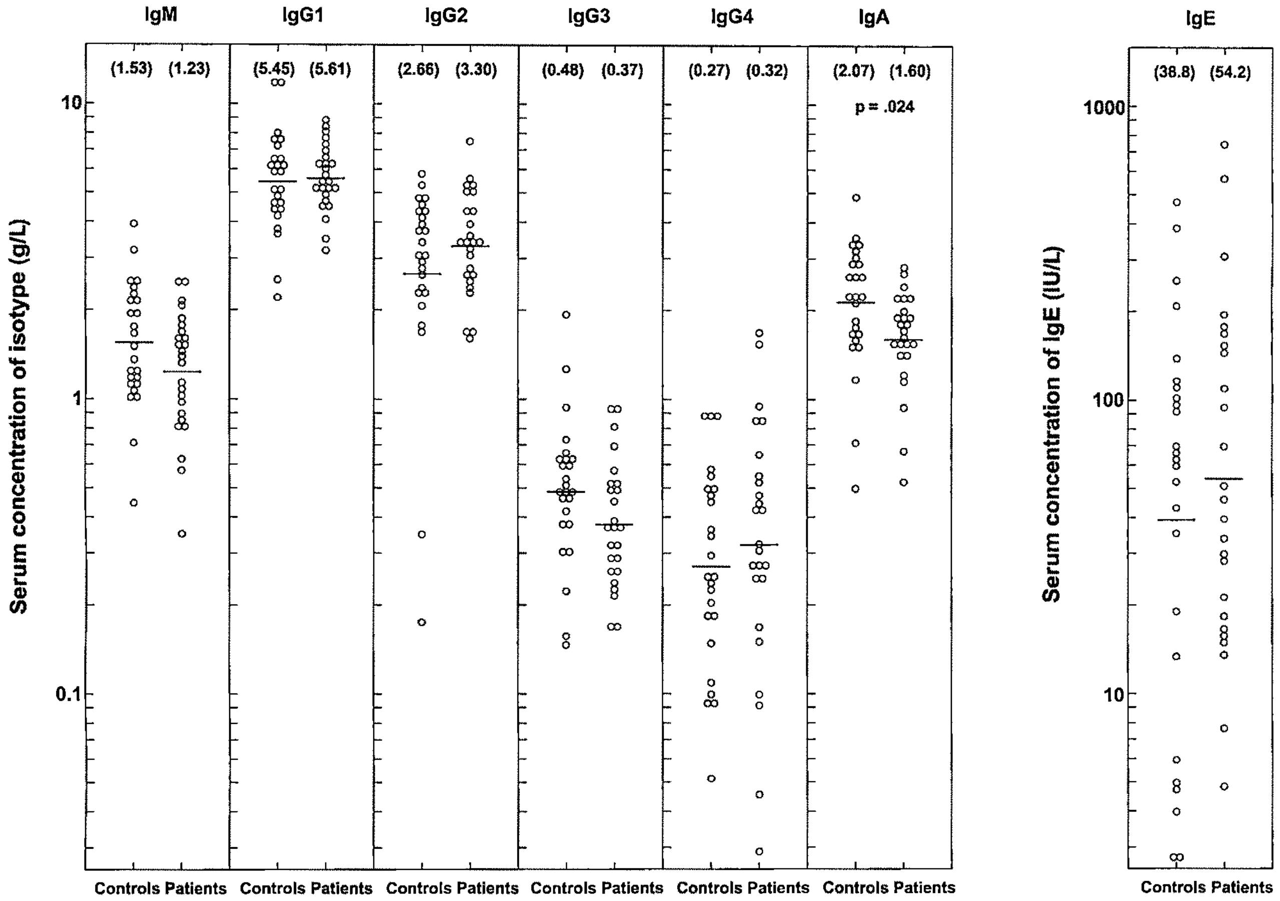

Fig 1. Serum concentrations of IgM, IgG1, IgG2, IgG3, IgG4, IgA, and IgE in 25 patients with recurrent sinusitis and in 25 age- and sex-matched healthy control individuals. Serum concentration of each isotype is plotted on y-axis. Geometric mean values are indicated by lines and are given in parentheses in grams per liter. Statistical significance (p = .024) of difference in geometric mean of IgA concentrations of patients and that of controls was calculated with Student's *t*-test of paired samples.

Polysaccharides and to Haemophilus influenzae *Type b Polysaccharide.* The concentrations of serum antibodies to bacterial polysaccharide antigens are presented in Fig 2. The mean concentration of IgG antibodies to pneumococcal type 14 polysaccharide was higher in the patients with sinusitis (2.54 μg/mL) than in the healthy control individuals (0.92 μg/mL, p = .008). In addition, in patients with sinusitis, a positive correlation existed between the concentration of IgG antibodies to type 14 polysaccharide and the serum concentration of IgA (Fig 3; Pearson product moment correlation coefficient r = .507, p = .01). In patients who had a concentration of serum IgA of less than 1.5 g/L (7 individuals), the geometric mean concentration of IgG antibodies to type 14 capsular polysaccharide was 0.94 μg/mL. In contrast, in patients who had a concentration of serum IgA of more than 2.0 g/L (7 individuals), the mean concentration of these antibodies was 4 times higher, or 4.0 μg/mL (Fig 3).

Female-to-Male Ratio in Patients Undergoing Endoscopic Nasal Surgery at Jorvi Hospital, Finland. Women were somewhat overrepresented (17 women among 25 patients, 68%) in the study group. To evaluate whether the female predominance was a general feature of the patients admitted to our hospital because of recurrent sinusitis, we analyzed the patient registry at Jorvi Hospital spanning the time period January 1997 to September 1999 (Table 3[4]). The registry was searched for the ICD-10 surgical procedure codes[4] DMB20 (endoscopic widening of the natural maxillary sinus ostium), DNB20 (endoscopic opening of ethmoid sinuses), and DHB20 (endoscopic nasal polypectomy). When a patient was operated on for the first time because of recurrent sinusitis at Jorvi Hospital, an endoscopic widening of the natural maxillary sinus ostium (DMB20) with or without opening of the ethmoid sinuses (DNB20) was performed. Of 340 such operations (DMB20 or DMB20 with DNB20), 188 operations were performed on women (55%, p = .0574, 2-tailed area of the normal distribution assuming that the female-to-male ratio is 1). If the patients required later further operations, an endoscopic opening of the ethmoid sinuses was usually performed (DNB20) without any surgery on the maxillary sinus ostium. Of these 35 operations (DNB20 only), 14 were performed on women (40%). Of the

TABLE 1. GEOMETRIC MEAN CONCENTRATIONS OF SERUM IGM, IGG1, IGG2, IGG3, IGG4, IGA, AND IGE IN 25 PATIENTS WITH RECURRENT SINUSITIS AND IN AGE- AND SEX-MATCHED HEALTHY CONTROLS

		Healthy Controls	*Patients*
Age (y)	Mean	44	44
	Range	19-64	19-64
Gender		18 F	18 F
		7 M	7 M
IgM (g/L)	Mean	1.53	1.23
	Range	0.45-4.04	0.35-2.50
	95% CI	0.59-4.00	0.48-3.18
IgG1 (g/L)	Mean	5.45	5.61
	Range	2.20-11.80	3.15-8.87
	95% CI	2.54-11.67	3.37-9.34
IgG2 (g/L)	Mean	2.66	3.30
	Range	0.17-5.73	1.58-7.40
	95% CI	0.55-12.97	1.52-7.18
IgG3 (g/L)	Mean	0.48	0.37
	Range	0.12-1.94	0.17-0.92
	95% CI	0.15-1.55	0.14-0.97
IgG4 (g/L)	Mean	0.27	0.32
	Range	0.05-0.90	0.03-1.71
	95% CI	0.06-1.25	0.05-2.25
IgA (g/L)	Mean	2.07	1.60
	Range	0.50-4.86	0.53-2.80
	95% CI	0.77-5.60	0.74-3.46
IgE (IU/L)	Mean	38.8	54.2
	Range	0.78-487	4.6-760
	95% CI	1.44-1050	4.1-723

Ninety-five percent confidence interval (CI) of assumed distribution of concentration values is based on estimate that logarithmic values of immunoglobulin concentrations follow normal distribution.

TABLE 2. FREQUENCY OF IGG3 HEAVY CHAIN CONSTANT REGION GENE ALLELES B AND G, IGG3 G3M(B) AND G3M(G) ALLOTYPES, IN PATIENTS WITH RECURRENT SINUSITIS

Genotype	*Finnish Population (Sarvas et al[8])*	*Controls*	*Patients*
b/b	74	7	8
b/g	103	15	9
g/g	30	3	7
Total	207	25	24
Frequency of G3m(g) allele	.394	.420	.479*

*No significant difference from controls.

60 polypectomy operations (DHB20), 21 were performed on women (35%, p = .0282, 2-tailed area of the normal distribution assuming that the female-to-male ratio is 1).

DISCUSSION

Patients with recurrent or chronic sinusitis have previously been reported to have alterations in serum IgG subclass concentrations. Decreased serum IgG2 concentrations have been found in children with recurrent sinopulmonary infections,[9] decreased serum IgG3 concentrations have been found in children[10] and adults with chronic sinusitis,[11,12] and increased IgG4 concentrations have been found in adult patients with chronic sinusitis.[12,13] Although the mean serum concentration of IgG3 was slightly lower and the mean concentration of IgG4 was slightly higher in our patients with sinusitis as compared with healthy individuals, the difference was not statistically significant.

The variability between the previously published results and our studies may reflect differences in the patient material. IgG2 deficiencies may be observed more frequently in children with recurrent sinusitis.[9] Increased IgG4 concentrations may reflect atopy as an etiologic factor of sinusitis, as in a previous report patients with chronic sinusitis and elevated IgG4 concentrations also had increased IgE concentrations and elevated peripheral blood eosinophil counts.[13] There may also be temporal variation in serum IgG subclass concentrations, as in some patients serum IgG3 concentrations were found to normalize over time.[13] Our failure to detect decreased concentrations of serum IgG3 in patients with sinusitis may be related to the fact that the serum samples were obtained from patients during a follow-up visit when they were relatively better off after surgical operations, or after recurrent drainage of maxillary sinuses and prompt antimicrobial therapy.

Serum IgG1, IgG2, and IgG3 concentrations are associated with polymorphisms in the immunoglobulin heavy chain constant region genes.[8] IgG3 concentrations are especially influenced by constant region gene polymorphism, as IgG3 allotype G3m(b) homozygote individuals have on the average 3-times-higher concentrations of IgG3 than IgG3 allotype G3m(g) homozygote individuals.[5,8,14,15] We were unable to detect statistically significant differences in IgG3 allotype gene frequencies in our patients with sinusitis as compared with healthy control individuals (Table 2). The results suggest that IgG3 G3m allotype status does not have a strong linkage to susceptibility to sinusitis.

Of the 25 patients, 17 were female (68%). This finding raised the possibility that the procedure for recruitment of the patients in our study resulted in a bias for female patients. Therefore, we wanted to analyze the sex distribution of all the patients operated on at our hospital. The analysis showed also a female preponderance in patients operated on because

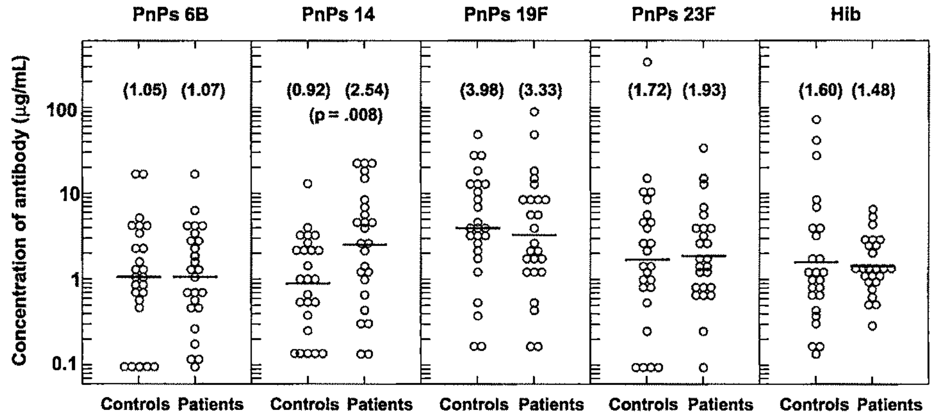

Fig 2. Serum concentrations of IgG antibodies to pneumococcal types 6B, 14, 19F, and 23F capsular polysaccharides (PnPs 6B, PnPs 14, PnPs 19F, and PnPs 23F, respectively) and concentrations of total antibodies to *Haemophilus influenzae* type b capsular polysaccharide (Hib) in 25 patients with recurrent sinusitis and in 25 age- and sex-matched healthy control individuals. Antibody concentrations are plotted on y-axis. Geometric mean values are indicated by lines and are given in parentheses in micrograms per milliliter. Statistical significance (p = .008) of difference in geometric mean of PnPs14 antibody concentrations of patients and that of controls was calculated with Student's *t*-test of paired samples.

of recurrent sinusitis. A female predominance in adult patients with recurrent or chronic purulent sinusitis has also been found in earlier studies. Of the 74 patients reported by Scadding et al,[12] 44 were female (59%); of the 30 patients reported by Armenaka et al,[11] 21 were female (70%); and of the 80 patients reported by Hoover at al,[13] 44 were female (55%). On the other hand, sinusitis that was associated with excessive mucosal reactions in the form of nasal polyposis or thickening of the ethmoid sinus mucosa appeared to be more common in men operated on at our hospital. The unequal sex distribution may reflect differences between the sexes in the activity of seeking surgical treatment, in the exposure to environmental factors leading to recurrent infections or to nasal polyposis, or in genetic composition.

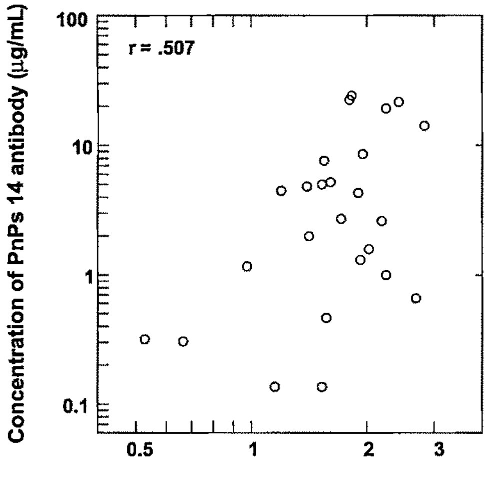

Fig 3. Serum concentration of IgA is plotted against serum concentration of antibodies to pneumococcal type 14 capsular polysaccharide (PnPs14) in 25 patients with recurrent sinusitis. Product moment correlation coefficient between serum IgA and PnPs14 antibody concentrations is given (r = .507, p = .01).

The mean concentration of IgA in patients with sinusitis was slightly lower (1.6 g/L) than the mean concentration of IgA in healthy control individuals (2.1 g/L, p = .024). None of our patients had a clear-cut IgA deficiency, but a few individuals with low IgA concentrations have been reported in earlier studies on patients with recurrent or chronic sinusitis.[9,11,13]

A decreased IgA serum concentration in patients with sinusitis may be related to a factor predisposing to sinusitis. Lack of IgA per se seems not to predispose to infections, as the majority of individuals with IgA deficiency are apparently healthy. However, the observation that patients with recurrent sinusitis have on the average decreased serum IgA concentrations is interesting in the context that a subpopulation of IgA-deficient individuals suffer from recurrent pyogenic infections.[16,17] IgA deficiency may share pathogenic features with common variable immune deficiency (CVID) with panhypogammaglobulinemia, as some individuals with IgA deficiency may later develop manifest CVID. This association is also supported by the observation that both of these diseases are associated with a locus between HLA class III minisatellite markers D821/D823 and HLA-B8, a DNA region populated by 21 genes that include tumor necrosis factor alpha and lymphotoxins alpha and beta, which have potential immunoregulatory functions.[18] The association between IgA deficiency and CVID raises the possibility that patients with recurrent sinusitis and a lower-than-average serum concentration of IgA may suffer from a mild form of a distinct immune deficiency disease that includes IgA deficiency and CVID at the most severe end of the disease spectrum. On the other hand, we cannot exclude the possibility that a decreased serum IgA concentration is a result of previous episodes of infection and is not related to a supposed preceding un-

TABLE 3. ENDOSCOPIC PARANASAL SINUS SURGICAL PROCEDURES PERFORMED IN JORVI HOSPITAL FROM JANUARY 1997 TO SEPTEMBER 1999

	Recurrent Sinusitis	*Reoperation Because of Mucosal Thickening*	*Nasal Polyposis*	*All Indications*
Surgical procedure	Widening of natural maxillary sinus ostium	Anterior ethmoidectomy	Polypectomy	
ICD-10 code[4] of procedure	DMB20 or DMB20 with DNB20	DNB20	DHB20	
Women	188 (55%)	14 (40%)	21 (35%)	223 (51%)
Men	152	21	39	212
All	340	35	60	435

Surgical operations are grouped on basis of indication for surgery. Percentage of women operated on within each group is given in parentheses. Women were more frequently operated on because of recurrent sinusitis (55%) as compared with frequency of operations performed because of excessive mucosal reactions in form of thickening of ethmoid sinus mucosa (40%) or nasal polyposis (35%; $\chi^2 = 10.34$, $df = 2$, $p < .01$).

derlying immune defect.

Patients with recurrent sinusitis had elevated levels of antibodies to pneumococcal type 14 capsular polysaccharide as compared with healthy control individuals. This finding suggests that some of the study patients had experienced infections caused by pneumococcus type 14 strains (Fig 2). However, pneumococcus type 14 strains have previously been reported to be relatively rare in sinusitis and in otitis media, which have usually been associated with pneumococcal type 19F, 6B, and 23F strains.[19,20] Pneumococcal type 14 strains have previously been found to be relatively frequent in invasive disease in Finland.[21] Therefore, it is possible that our patients experienced episodes of bacteremia caused by pneumococcus type 14 strains that then would have elicited antibody responses against the type 14 capsular polysaccharide. It may even be possible that pneumococcus type 14 infections may result in higher antibody responses to capsular polysaccharides than pneumococcus type 6B, 19F, and 23F infections. Immunogenicity studies on pneumococcal conjugate vaccines have revealed that vaccination resulted in relatively high antibody responses against type 14 capsular polysaccharide as compared to type 6B, 19F, and 23F polysaccharides.[22,23] Therefore, the observation that type 14 antibodies, but not type 6B, 19F, or 23F antibodies, were elevated may reflect the immunogenicity of type 14 polysaccharide. We previously found that pneumococcus type 14 strains were relatively infrequent (3 of 55 strains) among pneumococcal strains isolated from maxillary sinus effusions.[24]

The concentration of IgG antibodies to pneumococcal type 14 capsular polysaccharide had a positive correlation with the serum concentration of IgA in patients with sinusitis (Fig 3). High concentrations of IgG antibodies to pneumococcal type 14 polysaccharide were detected only in the patients with the highest serum IgA concentrations. This association raises the possibility that in a subpopulation of patients with recurrent or chronic sinusitis, a low serum IgA concentration may be associated with an impaired ability to mount antibody responses to polysaccharide antigens.

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Ann Otol Rhinol Laryngol 110:2001

EFFECTS OF CATECHOLAMINES ON CALVARIAL BONE RESORPTION IN VITRO

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Many important diseases in otolaryngology manifest through abnormal bone remodeling or destruction. The mechanisms for such pathological remodeling remain poorly understood. Bone is known to be innervated by norepinephrine-containing sympathetic nerves, and sympathectomy is known to induce bone resorption. The role, however, of norepinephrine as a potential bone-modulatory substance is unknown. Using the calvarial calcium release assay, we conducted the following experiment to evaluate the bone-modulatory activity of norepinephrine, the alpha-agonist octopamine, and the beta-agonist isoproterenol. Each agent was tested at 2 concentrations with and without parathyroid hormone. Norepinephrine was found to have no effect on calcium release. In contrast, octopamine at 10^{-8} mol/L exerted a significant *stimulatory* effect on calcium release, and isoproterenol at 10^{-6} mol/L exerted a significant *inhibitory* effect on parathyroid hormone–induced calcium release. The investigation suggests that a bimodal, concentration-dependent, receptor-specific model for catecholamine-mediated modulation of bone resorption may operate in calvarial bone.

KEY WORDS — bone resorption, isoproterenol, membranous bone, norepinephrine, octopamine.

INTRODUCTION

Abnormal remodeling or destruction of cranial bone is the pathological hallmark for numerous otolaryngological diseases, including chronic otitis media, cholesteatoma, otosclerosis, osteopetrosis, Paget's disease of bone, osteogenesis imperfecta, and fracture malunion.[1] The mechanisms of osteoclastic activation and bone resorption that underlie these disorders are poorly understood. Numerous factors have been implicated in the control mechanisms of bone metabolism, including prostaglandins (PGs),[2] leukotrienes,[3] interleukins,[4] tumor necrosis factor,[5] interferon,[6] transforming growth factors,[7] neuropeptides,[8] and physical strain.[9]

The sympathetic nervous system may also play a role in the control mechanisms of bone resorption. This linkage was first suggested by Fell,[10] who found surgical sympathectomy to significantly increase the haversian resorption spaces of feline long bones. Surgical sympathectomy was also shown by Sandhu et al[11] to increase osteoclast counts within the incisor root sockets of rat pups. A follow-up study by Sandhu et al[12] confirmed this observation and found the effect to be well established within 24 hours of sympathectomy. Pharmaceutical sympathectomy induced by guanethidine sulfate was described by Hill et al[13] to increase osteoclast counts in rat mandibles when evaluated 7 weeks after neonatal treatment. More recent investigations conducted by our laboratory have shown surgical sympathectomy (induced by the resection of ascending cervical sympathetic fibers), pharmaceutical sympathectomy (induced by guanethidine sulfate infusion), and chemical sympathectomy (induced by 6-hydroxydopamine injection) to similarly increase resorption within the middle ear bullae of adult Mongolian gerbils.[14-16]

The mechanism of sympathectomy-induced bone resorption is unknown. We previously hypothesized[16] that injury to sympathetic fibers (induced by sympathectomy) leads to *decreased* sympathetic uptake of nerve growth factor (NGF). Decreased NGF uptake by injured sympathetic fibers would thus result in an *increase* in NGF uptake by sensory fibers competing for NGF from end organ bone. This increase in sensory fiber NGF uptake would lead to increased sensory fiber stimulation and substance P (SP) release, with SP being a putative stimulator of osteoclastic resorption.[16-18]

However, an alternative mechanism of how sympathectomy might induce bone resorption may be related to the primary constituent of sympathetic nerves: norepinephrine (NE). Norepinephrine is the principal neurotransmitter of the peripheral sympathetic nervous system.[19] The transmitter has been shown by fluorescence microscopy, tyrosine hydroxylase immunohistochemistry, and dopamine beta-hydroxylase immunohistochemistry to be abundantly present within the sympathetic fibers innervating bone and

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periosteum.[8,20-23] These NE-containing fibers have been described as forming rich plexuses around bone vascular elements[8,20] and have been reported to co-label with the neuromodulatory protein neuropeptide Y (NPY).[8,21,22] Such co-localization of NPY with NE is not, however, unique to bone, as the transmitters have been found co-localized within sympathetic fibers innervating many viscera and blood vessels.[24,25]

In addition to being abundantly present within sympathetic fibers innervating bone, the neurotransmitter NE has been shown to modulate bone cell cyclic adenosine monophosphate (cAMP) levels in vitro.[21,26,27] For instance, NE, like parathyroid hormone (PTH), causes dose-dependent increases in cAMP formation in bone cells isolated from mouse calvaria and in immortalized bone cell lines such as Saos-2 (human osteosarcoma), MC3T3 (mouse calvarial), UMR-106-01 (rat osteosarcoma), and ROS 17/2.8 (rat osteosarcoma).[21,26] In contrast, NPY was found to truncate such NE-induced increases in cAMP levels in both UMR-106 and isolated mouse calvarial cells.[21,27]

Hence, NE is a neurotransmitter abundantly present within sympathetic fibers innervating bone and periosteum and is capable of modulating osteoblastic function through changes in cAMP levels. These observations imply that such a constitutively present sympathetic neurotransmitter may have the potential to alter local bone metabolism in vivo. In particular, local release of NE immediately after nerve resection (or damage) could be responsible for the early resorption-inductive effects seen with sympathectomy (thought by Sandhu et al[12] to occur immediately after axotomy). The following experiment was therefore designed to evaluate the effects of NE, the alpha-agonist octopamine (OCT), and the beta-agonist isoproterenol (ISO) on membranous bone resorption by means of the murine calvarial calcium release assay. In this way, we could assess the presence and specificity of the effects of catecholamines on calvarial bone resorption in vitro.

METHODS AND MATERIALS

Experimental Design. The effects of NE, the alpha-agonist OCT, and the beta-agonist ISO were determined on 72-hour calcium release from cultured neonate mouse hemicalvaria. Each agent was tested at 10^{-8} mol/L and 10^{-6} mol/L, with and without concomitant PTH, which served as the positive control (10^{-8} mol/L). The treatment combinations were independently assayed with 8 neonatal mouse hemicalvarial explants cultured in serum-enriched medium. After a 24-hour stabilization period, the bone explants were cultured for an experimental period of 72 hours, at the end of which, samples from the treatment and control media were analyzed for calcium concentration. The ability of a treatment to induce bone resorption or inhibit PTH-stimulated bone resorption was thus quantified as the net change in the incubation medium's calcium level as compared to that of controls.

Neurotransmitters and Agents. The adrenergic agents were purchased from Research Biochemicals Inc (Natick, Massachusetts) and used without further purification. Stock solutions of L (–)-norepinephrine bitartrate (NE; L(–)-4-(2-amino-L-hydroxyethyl)-1,2-benzenediol bitartrate); (±)-octopamine hydrochloride (OCT; (±)-alpha-(aminomethyl)-4-hydroxybenzyl alcohol hydrochloride); and *R*(–)-isoproterenol bitartrate (ISO; *R*(–)-4-[L-hydroxy-2-((1-isopropyl)amino)ethyl]-1,2-dihydroxybenzene) were freshly prepared before each dosing. Milligram aliquots of each agent were dissolved in sterile culture medium so that a 10-µL aliquot would bring the 2 mL of incubation medium per tube to a final concentration of 10^{-8} or 10^{-6} mol/L.

Synthetic bovine PTH was purchased from Sigma Chemical Co (St Louis, Missouri) and used without further purification. After we accounted for the purity of the sample lot, the hormone was reconstituted in sterile culture medium and refrigerated at 4°C. The stock solution of PTH was thus prepared so that 10 µL added to 2 mL of incubation medium would result in a final medium concentration of 10^{-8} mol/L.

Confirmatory studies were also conducted with the alpha-antagonist phenoxybenzamine (PBA; Research Biochemicals) and the cyclooxygenase inhibitor indomethacin (INDO; Sigma). Each was reconstituted in sterile culture medium before use so that 10 µL added to 2 mL of incubation medium would result in final medium concentrations of 10^{-8} mol/L for PBA and 5×10^{-7} mol/L for INDO.

Animals and Media. The protocol used in this investigation is a well-established modification of that of Goldhaber et al[28,29] as used in our laboratory.[30] Pregnant Swiss-Webster mice, timed to deliver 3 days before the study, were obtained from Simonsen Laboratories (Gilroy, California). Their use was approved by the Institutional Animal Care and Use Committee of the University of California, Davis. Three days after parturition, the weight of each neonate was recorded and the animals were placed into groups of 8. The groups were arranged to eliminate gross differences in the distributions of weights, and animals with weights of more than 1 g above or below the group mean were excluded. Animals of both sexes were used.

Four milliliters of incubation medium was required

SUMMARY OF ONE-WAY ANOVA WITH MULTIPLE COMPARISONS TESTS, PAIRED *T*-TESTS, AND TESTS FOR LINEAR TRENDS FOR CALCIUM RELEASE WITH LOW- AND HIGH-DOSE NE, ISO, AND OCT, WITH AND WITHOUT CONCOMITANT PTH STIMULATION

Comparison	t	*Bonferroni p*
NE at 10^{-8} and 10^{-6} mol/L without PTH versus base*		
Base (2.55 ± 2.64) versus NE (10^{-8}) (6.25 ± 3.86)	2.3131	>.05 (NS)
Base (2.55 ± 2.64) versus NE (10^{-6}) (1.18 ± 1.10)	0.8544	>.05 (NS)
NE at 10^{-8} and 10^{-6} mol/L with PTH versus base with PTH		
PTH (10^{-8}) (39.57 ± 4.69) versus NE (10^{-8}) with PTH (10^{-8}) (35.93 ± 10.10)	0.8589	>.05 (NS)
PTH (10^{-8}) (39.57 ± 4.69) versus NE (10^{-6}) with PTH (10^{-8}) (41.37 ± 6.09)	0.4255	>.05 (NS)
ISO at 10^{-8} and 10^{-6} mol/L without PTH versus base		
Base (10.77 ± 6.69) versus ISO (10^{-8}) (11.22 ± 5.00)	0.0731	>.05 (NS)
Base (10.77 ± 6.69) versus ISO (10^{-6}) (19.65 ± 16.46)	1.4434	>.05 (NS)
ISO at 10^{-8} and 10^{-6} mol/L with PTH versus base with PTH†		
PTH (10^{-8}) (48.77 ± 4.40) versus ISO (10^{-8}) with PTH (10^{-8}) (43.27 ± 4.65)	1.4401	>.05 (NS)
PTH (10^{-8}) (48.77 ± 4.40) versus ISO (10^{-6}) with PTH (10^{-8}) (37.00 ± 9.45)	3.0810	<.05
OCT at 10^{-8} and 10^{-6} mol/L without PTH versus base§		
Base (2.55 ± 2.64) versus OCT (10^{-8}) (6.83 ± 1.76)	3.2369	<.05
Base (2.55 ± 2.64) versus OCT (10^{-6}) (1.48 ± 2.39)	0.8061	>.05 (NS)
OCT at 10^{-8} and 10^{-6} mol/L with PTH versus base with PTH		
PTH (10^{-8}) (39.57 ± 4.69) versus OCT (10^{-8}) with PTH (10^{-8}) (48.57 ± 5.17)	3.3472	<.01
PTH (10^{-8}) (39.57 ± 4.69) versus OCT (10^{-6}) with PTH (10^{-8}) (36.15 ± 4.05)	1.2707	>.05 (NS)

ANOVA — analysis of variance, NE — norepinephrine, ISO — isoproterenol, OCT — octopamine, PTH — parathyroid hormone, NS — not significant.

*Repeat study with paired design, NE at 10^{-8} versus base, by paired Student's t: p = .087, t = 2.047, df = 6.

†Test for linear trends, column versus means: slope = −5.8833, r^2 = .3892, F = 9.5675, p = .0074.

§Repeat study with paired design, OCT at 10^{-8} versus base, by paired Student's t: p = .0005, t = 6.708, df = 6.

for each hemicalvarium: 2 mL for preincubation and 2 mL for the experimental period. The stock incubation medium was composed of 15% heat-inactivated horse serum (Sigma), 84% Dulbecco's modified eagle medium (Gibco Laboratories, Grand Island, New York), and 1% antibiotic-antimycotic lyophilized powder in 0.9% saline solution (Sigma). The horse serum was first "heat-inactivated" by submersion for 1 hour in a constantly stirred 59°C water bath. The antibiotic-antimycotic lyophilized powder was reconstituted with 20 mL of sterile culture medium and dispensed at 10 µL per milliliter of medium. The final antimicrobial concentrations were thus 100-U/mL penicillin, 0.1-mg/mL streptomycin, and 0.25-µg/mL amphotericin B.

Calvarial Culture Assay. Sets of 10 sterile Pyrex test tubes were filled with base medium and NE, OCT, or ISO at 10^{-8} mol/L or 10^{-6} mol/L with or without PTH at 10^{-8} mol/L. The 9th and 10th tubes of each set were refrigerated before incubation without the addition of bone samples, so that a baseline (preincubation) calcium level could be measured. Groups of 8 mice were euthanized in a carbon dioxide chamber, and their bodies were reweighed, submerged in ethanol, and air-dried under a sterile hood. Their frontoparietal calvaria were dissected, trimmed, and split into halves. These hemicalvarial explants were placed into the labeled test tubes containing 2 mL of medium and gassed with a mixture of 50% oxygen, 45% nitrogen, and 5% carbon dioxide (Liquid Carbonic Co, Chicago, Illinois). The tubes were then stoppered and placed into a rotating drum located within a humidity-controlled culture oven maintained at 37.5°C. After 24 hours, the initial medium was replaced with stock medium (2 mL per tube) plus the specific agent being tested with or without PTH. The tubes were then regassed and incubated for 72 hours, with regassing every 24 hours.

Calcium Analysis and Statistical Comparisons. Calcium concentrations (in micrograms per milliliter) were determined with a Perkin Elmer 3030 B atomic absorption spectrophotometer. Three samples from each tube were analyzed to give a mean calcium concentration per tube. The experimental and control calcium values were then compared as unmatched groups via 1-way analysis of variance with Bonferroni-corrected multiple comparisons post hoc tests (InStat 2.0 biostatistical program, GraphPad Software, San Diego, California). Confirmatory studies (when conducted) utilized paired and unpaired 2-tailed Student's *t*-tests. The significance for all comparisons was set at 95%.

RESULTS

Norepinephrine. Norepinephrine, both at 10^{-8} mol/L and at 10^{-6} mol/L, exerted no significant effect on calcium release as compared to bone incubated in control medium alone (p > .05; see Table and Fig 1,

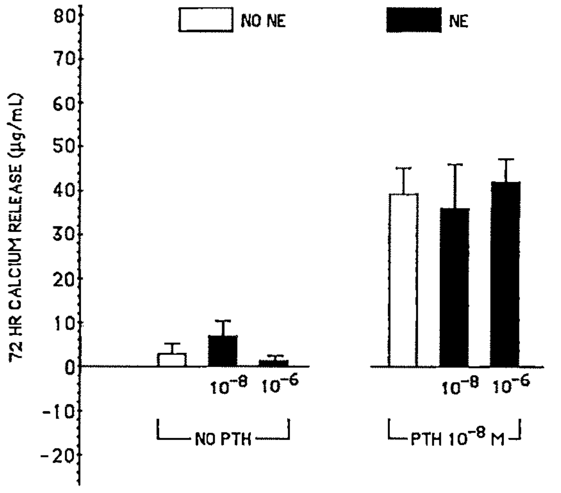

Fig 1. On left, bar graph compares effects of norepinephrine (NE) at 10^{-8} mol/L and 10^{-6} mol/L on unstimulated (no parathyroid hormone [PTH]) calcium release. Results show that NE exerted no effect as compared to saline-treated controls. On right are effects of NE on PTH-stimulated calcium release as compared to PTH-stimulated controls. No inhibitory effects were observed. Error bars represent standard deviations of means.

left graph). The experiment was then repeated with a paired hemicalvarial design (ie, left versus right) to allow reanalysis via the paired Student's *t*-test. These data again showed calcium release induced by NE at 10^{-8} mol/L to be nonsignificant as compared to calcium release by control medium alone ($p = .087$; see Table).

Norepinephrine was also found to have no effect on PTH-induced calcium release (see Table and Fig 1, right graph). The agent at 10^{-8} mol/L and at 10^{-6} mol/L exerted no significant effect on calcium release as compared to calcium release from PTH-stimulated controls ($p > .05$). Norepinephrine has thus been shown to have no stimulatory effect on calcium release, nor any inhibitory effect on PTH-stimulated calcium release.

Isoproterenol. Isoproterenol, both at 10^{-8} mol/L and at 10^{-6} mol/L, was found to have no significant stimulatory effect on calcium release as compared to control medium alone ($p > .05$; see Table and Fig 2, left graph). In contrast, ISO at 10^{-6} mol/L, but not at 10^{-8} mol/L, exerted a significant inhibitory effect on PTH-stimulated calcium release as compared to PTH alone ($p < .05$; see Table and Fig 2, right graph). Furthermore, a test for linear trends revealed a significant dose-response relationship between ISO and the inhibition of PTH-induced calcium release ($F = 9.57$, $r^2 = .389$, $p = .007$). The results thus show that ISO exerts no stimulatory effect on calcium release, while ISO at 10^{-6} mol/L, but not 10^{-8} mol/L, exerts a significant inhibitory effect on PTH-stimulated calcium release.

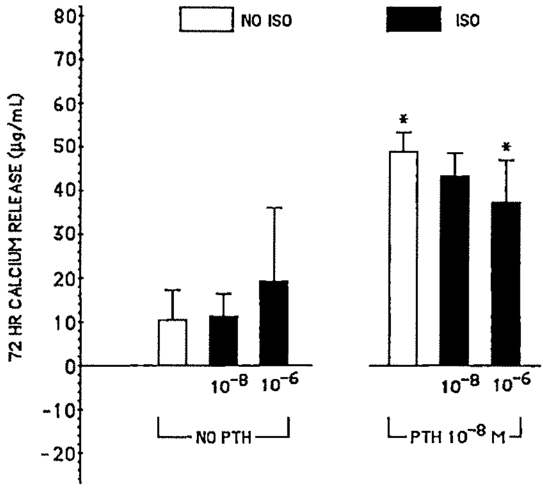

Fig 2. On left, bar graph compares effects of isoproterenol (ISO) at 10^{-8} mol/L and 10^{-6} mol/L versus unstimulated (no PTH) calcium release. Results show that ISO exerted no effect as compared to saline-treated controls. On right are effects of ISO on PTH-stimulated calcium release as compared to PTH-stimulated controls. Significant inhibitory effect was observed at 10^{-6} mol/L. Error bars represent standard deviations of means. Paired symbols denote differences at 95% confidence level.

Octopamine. Octopamine at 10^{-8} mol/L induced calcium release significantly greater than that of both the control medium ($p < .01$) and the medium containing OCT at 10^{-6} mol/L ($p < .05$; see Table and Fig 3, left graph). The experiment was repeated with a paired left-versus-right design to confirm the results. This study again showed OCT at 10^{-8} mol/L to significantly increase calcium release as compared to control medium alone ($p < .001$; see Table).

The robust stimulatory effect of OCT on calcium release was also evident in the PTH-stimulated group (see Table and Fig 3, right graph). Post hoc multiple comparisons analysis showed the net calcium release from calvaria incubated with PTH and OCT at 10^{-8} mol/L to be significantly greater than that of bone incubated with PTH and OCT at 10^{-6} mol/L ($p < .001$) or PTH alone ($p < 0.01$). No evidence for an inhibitory effect on PTH-stimulated release was observed. Alpha-agonism with OCT, but only at the low concentration of 10^{-8} mol/L, has thus been shown to exert a significant stimulatory effect on calcium release, both alone and in the presence of 10^{-8} mol/L PTH.

With the effect of OCT being seen only at 10^{-8} mol/L (ie, at a concentration potentially physiologic for the direct sympathetic modulation of bone remodeling), an additional set of studies were conducted to further confirm and characterize this effect. Hence, the alpha-antagonist PBA and the cyclooxygenase inhibitor INDO were employed to verify the receptor specificity of the effect and to determine whether the effect was PG-dependent. Net calcium releases

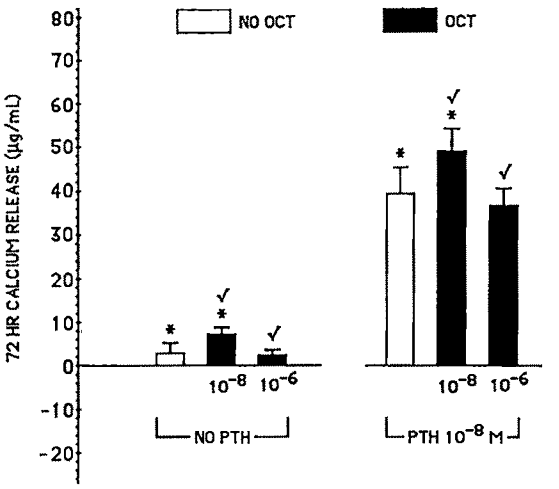

Fig 3. On left, bar graph compares effects of octopamine (OCT) at 10^{-8} and 10^{-6} mol/L on unstimulated (no PTH) calcium release. Results show that OCT induced calcium release, but only at 10^{-8} mol/L, as compared to saline-treated controls. On right is effect of OCT on PTH-stimulated calcium release as compared to PTH-stimulated controls. No inhibitory effect was observed, although significant increase was seen in OCT at 10^{-8} mol/L plus PTH group as compared to PTH-alone group or compared to OCT at 10^{-6} mol/L plus PTH group. Error bars represent standard deviations of means. Paired symbols denote significant differences at 95% confidence level.

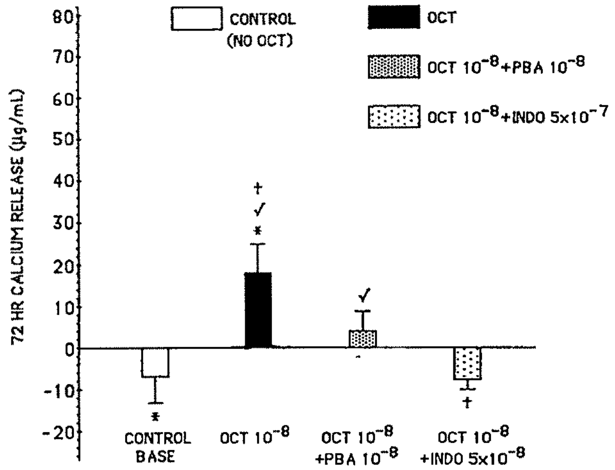

Fig 4. Effects on 72-hour calcium release of OCT at 10^{-8} mol/L with and without alpha-antagonist phenoxybenzamine (PBA) at 10^{-8} mol/L or prostaglandin inhibitor indomethacin (INDO) at 5×10^{-7} mol/L. Results show that OCT induced significant calcium release as compared to control medium, while PBA and INDO significantly inhibited effects of OCT. Negative values represent net uptake of calcium from incubation medium after 72 hours. Error bars represent standard deviations of means. Paired symbols denote significant differences at 95% confidence level.

induced by OCT, OCT plus PBA, and OCT plus INDO were compared with that of saline-only controls by 1-way analysis of variance with Bonferroni-corrected post hoc tests (Fig 4). These results show that calcium release from bone incubated with OCT at 10^{-8} mol/L was significantly greater than that from bone incubated in control medium ($p < .001$). The addition of either INDO at 5×10^{-7} mol/L or PBA at 10^{-8} mol/L significantly inhibited OCT-stimulated calcium release as compared to the OCT-stimulated group ($p < .001$ and $p < .01$, respectively). Negative values indicated the net uptake of calcium by cultured bone during the incubation period. The results thus show that OCT at 10^{-8} mol/L exerts a significant stimulatory effect on calcium release that is blocked by alpha-antagonism with PBA and eliminated via cyclooxygenase inhibition with INDO.

DISCUSSION

This experiment was conducted in a stepwise fashion to determine whether NE modulates calcium release from cultured calvarial bone in vitro. The experiment utilized the neonate calvarial calcium release assay, a model that allows for the evaluation of substances ex vivo in the absence of confounding vascular or systemic effects. Neonate bone is used in this assay because quiescent adult bone lacks the metabolic activity required for sufficient test sensitivity. Moreover, because results obtained with the neonate calvarial calcium release assay have correlated well with results from in vivo studies on adult subjects, the assay has become a well-established and commonly used vehicle for the investigation of disease processes in adult bone.

Therefore, by means of the calvarial calcium release assay, the catecholamine NE was found to have no significant stimulatory or inhibitory effect on calcium release after initial and repeat studies. Nevertheless, further inspection of the data suggested that concomitant agonism and antagonism of calcium resorption by NE may have contributed to this insignificance. This conclusion was supported by the second experiment, which showed beta-agonism with ISO to *inhibit* calcium release in a dose-dependent manner at 10^{-6} mol/L, and by the third experiment, which showed alpha-agonism with OCT to *stimulate* calcium release, but only at the low concentration of 10^{-8} mol/L. The significant inductive effects of alpha-agonism at 10^{-8} mol/L were then confirmed by repeat experimentation with a paired left-versus-right design. Finally, the calcium release induced by OCT at 10^{-8} mol/L was successfully blocked by alpha-antagonism with PBA at equimolar concentrations and via cyclooxygenase inhibition by INDO.

The results of this investigation thus support a possible bimodal, concentration-dependent, receptor-specific model for catecholamine-induced modulation of bone resorption. They suggest that NE may be capable of inducing bone resorption at physiological concentrations via an alpha-adrenergic receptor-mediated process that is mechanistically dependent on PG release. At higher concentrations, a dose-dependent inhibitory effect, mediated by beta-stimulation, may also be present. This conclusion is based not only on the contradictory results obtained for NE and the inhibitory effect of ISO, but on the general robustness displayed by OCT throughout the experiment (Fig 3).

The finding that OCT-induced resorption occurred at 10^{-8} mol/L and not at 10^{-6} mol/L may also have important implications for the putative role of NE as a physiological modulator of bone metabolism. The concentration of 10^{-8} mol/L was chosen to approximate physiologic concentrations of released neurotransmitter. The effect at 10^{-8} mol/L therefore implies that localized resorption may indeed be induced in vivo through the release of NE from peripheral sympathetic nerves. One could speculate that such a phenomenon might contribute to the creation of nutrient avenues during bone neovascularization, or that it might be associated with remodeling that occurs proximal to vascularized haversian canals.

While OCT at 10^{-8} mol/L induced significant calcium release, OCT at the higher concentration of 10^{-6} mol/L did not. This paradoxical response to increasing alpha-agonism may have been due to the effect's apparent dependence on PG release. For example, this investigation showed that OCT-induced resorption at 10^{-8} mol/L is blocked by INDO, so that a PG-dependent mechanism is likely. Previous investigations have shown PGE2 to *induce* bone resorption in organ culture through osteoblastic stimulation,[31,32] while it *inhibits* the activity of isolated osteoclasts in vitro.[33,34] One explanation for the differential effects displayed by alpha-agonism at 10^{-8} mol/L (stimulatory) versus 10^{-6} mol/L (nonstimulatory) may thus be that altered PG levels at higher agonist concentrations lead to the paradoxical inhibition of osteoclastic function.

This investigation's finding of a potential bone-modulatory role for NE is supported by the literature. The presence of NE, as indicated by tyrosine hydroxylase- and dopamine beta-hydroxylase–immunoreactive fibers, has been demonstrated within periosteum,[8,23] in developing heterotopic bone,[21,35] and in mature bone.[8,20-22] The transmitter has been shown to increase cAMP levels in osteoblast-like UMR 106 cells in a manner similar to that of PTH,[21,26,27] and the effects exerted by both PTH and NE on those cells have been shown to be equally inhibited by NPY.[21,27]

The observation that adrenergic stimulation is linked to PG release is supported by similar findings in other models. In the dog spleen, for example, nerve stimulation results in marked PGE2 release from capsular smooth muscle cells in a manner inhibited by alpha-antagonism with PBA.[36] Similarly, isolated and perfused rat kidneys have been shown to release PGE2 after intra-arterial administration of NE — a response that has been characterized as alpha-1–specific.[37] In vitro studies conducted with Madin-Darby canine kidney cells have also revealed NE to stimulate PGE2 release via an alpha-1–specific mechanism.[38] It has been proposed that in these instances of NE-induced, alpha-1–receptor–mediated PG release, receptor binding by NE results in the mobilization of intracellular and extracellular calcium stores with subsequent activation of calmodulin-dependent phospholipases, probably of the A2 class.[37]

Alpha-adrenergic stimulation is thus known to induce PG release in a variety of tissues. In bone, PGs, particularly PGE2, have been reported to increase resorption both in vivo[39-41] and in vitro.[31,42-44] The effects are thought to be mediated by osteoblasts that, upon stimulation by PG, increase their levels of cAMP.[45] Such a cAMP-mediated stimulatory mechanism is also thought to be utilized by PTH.[33,46] Indeed, PTH's resorptive effects may be enhanced by its induction of osteoblastic PG release, as such PGs further increase osteoblastic activity through autocrine stimulation.[47,48]

The data of this experiment therefore suggest that an antagonistic, concentration-dependent relationship may exist between alpha-adrenergic stimulation leading to the induction of bone resorption and beta-adrenergic stimulation leading to the inhibition of bone resorption. At low concentrations, alpha-mediated inductive effects are observed, while at higher concentrations, inhibitory beta-mediated effects are seen. One explanation for this observation may be that bone cells (ie, osteoblasts) bind NE via alpha-adrenergic receptors, leading to PG release at a local site, and that PG release leads to the activation of osteoclastic resorption. In contrast, such alpha-mediated effects may be limited by beta-mediated inhibition. The distribution and expression of alpha- and beta-receptors and their affinity for NE would therefore play significant roles in determining an inductive or inhibitory response to NE. Such a putative role of NE, in both normal and pathological skull bone metabolism, and NE's apparent effects on PG release require further investigation.

CONCLUSIONS

Norepinephrine, with both alpha- and beta-mediated adrenergic effects, exhibited no significant inhibitory or stimulatory activity in vitro. This lack of effect may have been due to the concomitant agonism and antagonism of resorption mediated by a concentration-dependent, receptor-specific mechanism. This hypothesis was confirmed when OCT at 10^{-8} mol/L, but not at 10^{-6} mol/L, was found to exert a significant *stimulatory* effect on calcium release, while ISO at 10^{-6} mol/L, but not at 10^{-8} mol/L, was shown to exert a significant *inhibitory* effect on PTH-induced calcium release. The inductive effect of alpha-agonism at 10^{-8} mol/L was further confirmed by blockade with the alpha-antagonist PBA at equimolar concentrations. Furthermore, alpha-agonist–induced calcium release was shown to be PG-dependent via inhibition with INDO. These data support previous investigations that have independently demonstrated links among NE, PG release, and bone resorption. Nevertheless, while NE-induced resorption closely linked to bone vascular elements *may* be of consequence, the role of NE in the induction of physiological and pathological bone resorption in the skull and facial skeleton is unknown.

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PENICILLIN TREATMENT FAILURE IN GROUP A STREPTOCOCCAL TONSILLOPHARYNGITIS: NO GENETIC DIFFERENCE FOUND BETWEEN STRAINS ISOLATED FROM FAILURES AND NONFAILURES

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Despite penicillin (pcV) treatment, tonsillopharyngitis caused by group A streptococci (GAS) is associated with bacterial failure rates as high as 25%. The reason for this rate of failure is not fully understood. One explanation might be that certain DNA profiles of GAS strains are responsible for treatment failures. Using arbitrarily primed polymerase chain reaction (AP-PCR), we compared the DNA profiles of GAS strains from 4 patients with several treatment failures following pcV treatment of tonsillopharyngitis with the profiles of strains of the same T type from patients who were clinically and bacteriologically cured after a single course of pcV. The isolates were obtained during the same time period and from the same geographic area. Thirty-seven strains of T types 4, 12, and R28 were investigated. Eleven different DNA profiles could be detected with the AP-PCR technique. Five DNA profiles were identified as T type 12, 3 as T type 4, and 3 as T type R28. The DNA profiles of the strains from the 4 patients with several treatment failures differed, but all isolates from each one of these patients exhibited the same or a very similar profile. The DNA profiles of the failure strains were also represented in nonfailure strains. Treatment failure in these 4 patients therefore seems to be due to insufficient eradication of GAS, rather than to reinfection with a new strain. The finding that the same DNA profile can be present in both failure and nonfailure strains suggests that the treatment failure may be to some extent host-related and not only due to bacterial factors.

KEY WORDS — arbitrarily primed polymerase chain reaction, penicillin, streptococci, tonsillopharyngitis, treatment failure.

INTRODUCTION

Group A streptococci (GAS) are the major bacterial cause of tonsillopharyngitis, accounting for 30% to 50% of cases.[1] Since, in untreated cases, the acute episode may develop into rheumatic fever or glomerulonephritis, antibiotic treatment of GAS tonsillopharyngitis is indicated.[2,3] Group A streptococcal tonsillopharyngitis treated with phenoxymethylpenicillin (pcV) has been associated with clinical and bacterial treatment failures in 10% to 25% of cases.[4,5] So far, there have been no reports on resistance against pcV in clinical GAS strains, but pcV-induced selection of β-lactamase–producing bacteria in the throat inactivating pcV is a possibility.[6,7] Penicillin tolerance, ie, delayed killing of GAS by pcV, has also been proposed to account for the failures,[8] although other studies have not been able to confirm this.[9] Low penetration of pcV into the site of infection,[10] the ability of GAS to invade epithelial cells,[11] and eradication of highly pcV-sensitive viridans streptococci that may inhibit GAS[12] might contribute to the lack of therapeutic success. Viridans streptococci are, however, also highly sensitive to clindamycin, which is superior to pcV in eradicating GAS from the throat.[5] More apparent explanations for treatment failures are poor patient compliance and reinfection from the environment. Another explanation might be that certain strains of GAS are responsible for treatment failures.

The aim of the present study was to explore the DNA polymorphism of GAS strains by arbitrarily primed polymerase chain reaction (AP-PCR), to determine whether bacterial treatment failures were due to insufficient eradication of the organism or reinfection with a new GAS strain, and to investigate whether isolates from patients with treatment failure differed genetically from isolates obtained from patients who were cured after 1 course of pcV who lived in the same geographic area during the same time period.

MATERIALS AND METHODS

PATIENTS

Patients were enrolled from a previous study in which the efficacy of pcV and clindamycin to eradicate GAS from the throat in patients with bacterial treatment failure after pcV treatment was compared.[13] The study was approved by the Medical Ethics Committee of the University of Lund. A total of 239 patients with GAS tonsillopharyngitis were treated with pcV for 10 days (12.5 mg/kg body weight twice per

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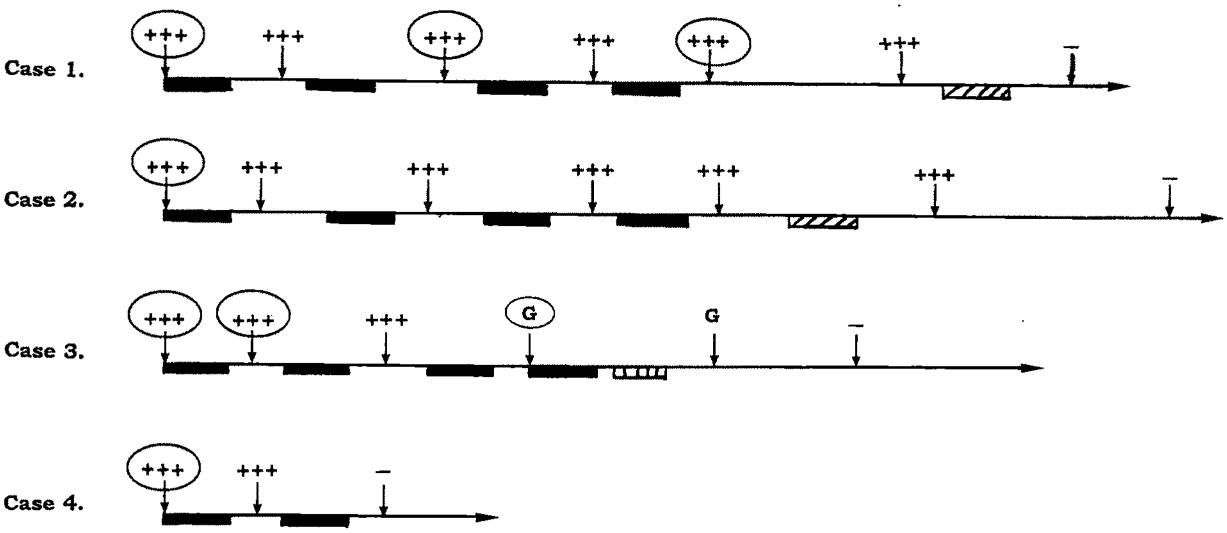

Fig 1. Outline of treatments. Oval — symptoms and signs of pharyngotonsillitis; solid bar — 10 days of penicillin treatment; slanted-hatched bar — 10 days of clindamycin treatment; upright-hatched bar — 7 days of trimethoprim-sulfamethoxazole treatment; +++ — abundant growth of group A streptococci; G — growth of group G streptococci; minus sign — negative throat culture.

day). At follow-up, usually 4 days but no later than 14 days after completing the treatment, 53 patients manifested bacterial treatment failure, ie, the presence of GAS of the same T type as in the original isolate. Of 48 evaluable patients with bacterial treatment failure, regardless of symptoms, 22 patients were treated with a second course of pcV and 26 patients with clindamycin hydrochloride (6.5 mg/kg body weight 3 times per day in children; 300 mg 3 times per day in adults). Persons in the patient's environment with symptoms and a positive culture for GAS were treated with pcV. After the second treatment, 64% (14/22) of the patients treated with pcV showed bacterial treatment failure, as compared to none of the 26 patients in the clindamycin group ($p < .001$, χ^2 test). The patients were followed for 1 year after inclusion, undergoing clinical examinations and throat cultures every third month and whenever signs of a sore throat appeared. Patient compliance was checked by a thorough interview of the patients and/or parents. It was emphasized that bacterial failure alone was the reason for further treatment.

Case 1. A 6-year-old boy was treated with a total of 4 pcV courses for growth of GAS of T type 4 in repeated throat cultures (Fig 1). On 3 of the occasions, he displayed clinical signs and symptoms of tonsillopharyngitis. Nobody in the patient's closest surroundings had any symptoms of a sore throat. The parents assured us that he had taken pcV according to the prescription all 10 days. Finally, clindamycin was prescribed. At the follow-up visits at 14 days and 1 and 3 months, the patient was asymptomatic and the throat cultures were negative.

After we performed AP-PCR, the DNA profiles of the 6 isolates of T type 4 from the boy were compared with the profiles of 7 strains of the same T type. (All cultures had abundant growth.) These latter strains were isolated during the same time period from randomly selected patients who were treated with a single course of pcV for GAS tonsillopharyngitis and who lived in the same geographic area as the boy. All 7 patients were successfully treated, both clinically and bacteriologically. The mean age of the patients was 9.1 years (SD, ±2.5 years).

Case 2. A 2-year-old boy with clinical tonsillopharyngitis and growth of GAS of T type 12 was treated with pcV for 10 days (Fig 1). The boy displayed no symptoms or signs of clinical tonsillopharyngitis at any of the 3 follow-up visits. As in case 1, the parents assured us that their son had taken the pcV according to the prescription, and nobody in the immediate surroundings had any symptoms of a sore throat. Clindamycin treatment was started. At the follow-up visit 20 days after the antibiotic treatment was terminated, the patient was asymptomatic, but still had abundant growth of GAS of T type 12 in the throat culture. No further antibiotics were given. At follow-up visits 6 weeks and 3 months later, throat cultures were negative for GAS.

The DNA profiles of the 6 isolates of T type 12 from the boy were compared with 7 strains of T type 12. (All cultures had abundant growth.) The latter 7 strains were selected as above. The mean age of the patients was 4.1 years (SD, ±1.7 years).

Cases 3 and 4. A 28-year-old mother and her 3-year-old son displayed clinical tonsillopharyngitis and growth of GAS of T type R28 (Fig 1). They were both treated with pcV for 10 days. At the follow-up visit within 6 days after completed treatment, the boy had no clinical symptoms, but had growth of GAS with the same T type as earlier. He was given a second pcV course. At the following control and 3 months later, the patient was still asymptomatic, and the throat cultures were negative. In contrast, the mother was prescribed 3 additional pcV courses because of repeated growth of the same T type (twice) followed by a positive culture for group G streptococci. At this point, she was treated with trimethoprim-sulfamethoxazole for a urinary tract infection. After completion of the antibiotic treatment, the throat culture was still positive for group G streptococci, but there were no clinical signs of tonsillopharyngitis. No further treatment was given. At follow-up visits after 3 weeks and 3 months, the mother had negative throat cultures and no clinical signs of tonsillopharyngitis. Nobody in the patients' closest surroundings had any symptoms of a sore throat. The mother assured us that she and her son had taken the pcV according to the prescription.

The DNA profiles of the 5 isolates of T type R28 from the mother and son were compared with 6 isolates of T type R28 (all with abundant growth) selected as above. The mean age of the patients was 9.7 years (SD, ±3.0 years).

BACTERIOLOGICAL INVESTIGATION

Culture specimens were obtained from the throat by rotating a sterile cotton swab along both tonsils. The specimens were transported in modified Stuart agar medium, inoculated onto horse-sheep blood agar, and incubated anaerobically for 18 to 20 hours. According to the number of β-hemolytic colonies, growth was classified semiquantitatively as sparse (1 to 10 colony-forming units [CFU]), moderate (10 to 100 CFU), or abundant (>100 CFU). The β-hemolytic streptococci were diagnosed by bacitracin susceptibility and enhanced hemolysis on horse blood agar by sodium nucleate, or serologically grouped.[14] Strains were T-typed by slide agglutination (Chemapol, Prague, Czech Republic). Antibiotic susceptibility against erythromycin, clindamycin, and doxycycline was determined by disk diffusion test (Oxoid Ltd, Basingstoke, England) on Iso-sensitest agar supplemented with 5% horse blood (Oxoid Ltd).

To determine the genetic profiles of the GAS strains, we carried out AP-PCR. The preparation of DNA was a modification of the method described by Jackson and Cook.[15] Briefly, the GAS strains were grown to an optical density of about 1.0 at 550 nm in Todd-Hewitt broth (Difco Laboratories, Detroit, Michigan). Each suspension was centrifuged, washed with 1XTE (40-mmol/L Tris–hydrochloric acid, pH 8.3, and 10-mmol/L ethylenediaminetetraacetic acid [EDTA], pH 8.3), and centrifuged again. The pellet was mixed with agarose (2.5% Low melt, Appligene, Saveen Biotech AB, Malmö, Sweden) and fluid paraffin, which had both been kept at 42°C. The mixture was vortexed and thereafter put on ice. After repeated washings with 1XTE, the resulting agarose pellets were centrifuged. The supernatant was removed, and 1 mL of 1XTE, 10 μL of proteinase K (20 mg/mL, Appligene), and 50 μL of 10% sodium dodecyl sulfate were added. After 1 hour of incubation (minimum) at 55°C, the DNA-containing pellets were cooled and washed. The pellets were kept at 4°C until used. One microliter of each DNA preparation was amplified by PCR in 10 μL of a reaction mixture containing 50-mmol/L Tris–hydrochloric acid (pH 8.3), 250-μg/mL bovine serum albumin, 2% (w/v) sucrose, 0.1-mmol/L cresol red, 3-mmol/L magnesium chloride, 200-μmol/L of each deoxynucloside triphosphate (Pharmacia Biotech, Uppsala, Sweden), 0.5-μmol/L of 1 of the 3 primers, and 0.6 U of Taq polymerase (Appligene). The primers included in the study were H2LM (CCTCCCGCCACC, DNA technology, Aarhus, Denmark), A70-9 (TGCAGCACCG, Genosys Biotechnologies Inc, Houston, Texas), and A70-10 (CAGACACGCC, Genosys). The mixture was processed for 45 cycles in a Rapid cycler (Idaho Technology, Idaho Falls, Idaho) with the following cycling parameters: 2 seconds of denaturation at 94°C, 30 seconds of annealing at 40°C, and 30 seconds of extension at 72°C. The reaction was finally extended for 5 minutes at 72°C. The whole product was electophoresed through a 1.5% agarose gel (Saveen Agarose Standard Molecular Biology Grade, Saveen Biotech AB) with ethidium bromide in Tris-borate-EDTA buffer (pH 8.0). The DNA bands were visualized under UV light and photographed. The sizes of the PCR products were compared with a DNA ladder (Life Technologies AB, Boehringer Mannheim, Mannheim, Germany). To check the reproducibility, we ran the samples twice.

RESULTS

The reproducibility of the AP-PCR was high. No differences in the number of bands or the relative intensity of bands could be discerned between assays. The method also demonstrated a high discriminatory capacity. In contrast to the 3 types recognized by the conventional T typing, 11 different DNA profiles could be detected with the AP-PCR technique. The DNA profiles had the following T type distribution: 5 DNA profiles were classified as T type 12, 3 as T type 4, and 3 as T type R28. All 37 GAS strains were susceptible to bacitracin, erythromycin, clindamycin, and doxycycline.

Case 1 showed the same DNA profile in 5 of 6

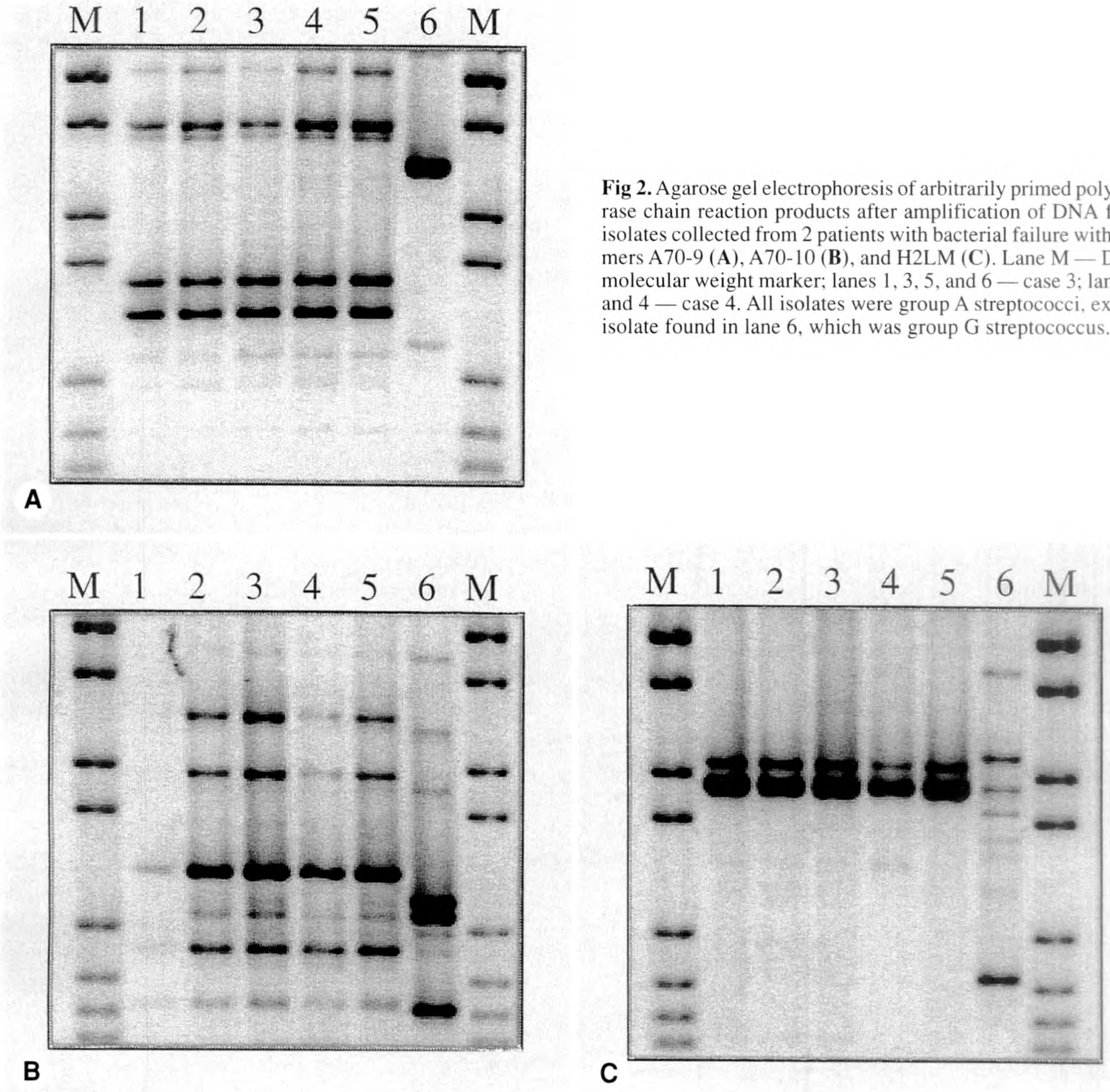

Fig 2. Agarose gel electrophoresis of arbitrarily primed polymerase chain reaction products after amplification of DNA from isolates collected from 2 patients with bacterial failure with primers A70-9 (**A**), A70-10 (**B**), and H2LM (**C**). Lane M — DNA molecular weight marker; lanes 1, 3, 5, and 6 — case 3; lanes 2 and 4 — case 4. All isolates were group A streptococci, except isolate found in lane 6, which was group G streptococcus.

isolates, which were all of T type 4. The isolate that was genetically not completely identical with the other isolates recovered from this patient was obtained after the fourth pcV course. This isolate had an extra band of high intensity and a size of about 1,200 base pair (bp). The extra band was present after repeated PCR runs, but it could not be detected after the fifth pcV course, when the original band pattern reappeared. The DNA profile found in all but 1 isolate from this patient was not unique. The same DNA profile was found in 4 of the 7 T type 4 strains from patients with no bacterial treatment failure. The remaining 3 nonfailure strains of the same T type were assigned to 2 different clones.

The isolates from case 2 were identified as T type 12, and the genetic profiles of these isolates were identical (6/6). This clone was also represented in 2 of the 7 nonfailure strains. The other 5 nonfailure strains of T type 12 exhibited band patterns corresponding to 4 different clones.

Cases 3 and 4, mother and son, exhibited the same genetic profile in all 5 isolates classified as type R28 (Fig 2). This profile was found in 4 of the 6 nonfailure strains, whereas 2 of the 6 had unique genetic profiles in the material investigated.

DISCUSSION

The present study showed that the isolates of GAS from the 4 patients with several treatment failures after a course of pcV treatment exhibited the same

DNA profiles. This excluded infection by a new GAS strain and is in accordance with the findings of Österlund and Engstrand,[16] who used AP-PCR for investigating GAS strains before and after antibiotic treatment. Bingen et al[17] used restriction length polymorphism of total DNA and also found most pretreatment and posttreatment strains to have the same genetic profile. However, the present study also investigated strains from persons in the same geographic area without relapses, as well as members of the same family. The persons in the patients' environment in our study were treated with pcV in the case of symptoms and positive culture for GAS; hence, the patients were probably not reinfected with the same strain from their immediate surroundings. More likely, the original GAS strain was not totally eradicated by the pcV. Since the number of β-lactamase–producing bacteria increases during pcV treatment, inactivation of pcV might explain the failure to eradicate GAS.[7] However, Tanz et al[18] did not find any differences between pcV and amoxicillin with clavulanic acid in eradicating GAS, and in a recent study[19] comparing tonsillar core cultures from patients with recurrent GAS tonsillitis and controls suffering from sleep apnea with no histories of tonsillitis, there was no difference in the presence of β-lactamase–producing bacteria. It has been reported that GAS might hide intracellularly in epithelial cells,[11] and this might be one explanation of the failure of the pcV treatment. The GAS are located predominantly on the surface and in the crypts of the tonsils,[20] and pcV penetrates only to the tonsillar surface fluid during the first days of infection.[10] This finding might explain the better eradication of GAS by clindamycin,[5,13] which penetrates better into the site of infection.[21]

The isolates of GAS obtained from patients after 1 successful pcV course showed the same DNA profile as those obtained from the patients with several treatment failures. Also, the isolates were from the same geographic area and were isolated during the same period of time. These similarities seem to exclude the possibility that special strains could account for the treatment failures. With the available new DNA techniques, the ability to differentiate strains is very good. A single mutation can therefore be discovered. In case 1, the extra band found in 1 of the isolates was probably due to 2 bacterial populations of GAS.

The mother and son had the same DNA profiles of GAS. The son was cured after 2 pcV courses, whereas the mother had 3 pcV courses and then suffered another attack of tonsillitis with group G streptococci, which necessitated 2 more pcV courses. This outcome implies that recurrent tonsillitis is probably a host problem and is not due to a particular GAS strain. In a study by Norgren et al[22] using the PCR technique, the same DNA profile of GAS of T type 1 was present in patients with bacteremia as in asymptomatic carriers in the same family; this finding suggests individual differences in immunity.

The isolates from the patients with repeated treatment failure were not always correlated to clinical symptoms and signs of tonsillitis. Obviously, only symptomatic patients with clinical tonsillopharyngitis should be treated with antibiotics. The study from which the patients were enrolled, however, was based on objective data, ie, cultures, and the purpose of this former study was to eradicate GAS from the throat.[13] The boy in case 2 had clinical tonsillopharyngitis only on the first visit and probably represented asymptomatic carriership.

CONCLUSION

To summarize, in this study, certain DNA profiles of GAS did not seem to be responsible for bacterial treatment failures, since both failure and nonfailure strains could exhibit the same DNA profile. In the 4 patients with treatment failure, the DNA profiles of the posttreatment isolates corresponded to those of the original pretreatment strains. Furthermore, even though isolates from 2 members of the same family yielded identical DNA profiles, the outcome of pcV treatment differed for these 2 patients. Treatment failure in GAS tonsillopharyngitis may therefore be associated with host factors, rather than bacterial factors.

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IMAGING CASE STUDY OF THE MONTH

TRANSTYMPANIC PENETRATING INJURY TO THE INTERNAL AUDITORY CANAL

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INTRODUCTION

Penetrating stab wounds of the ear are potentially devastating injuries. We report a case of a penetrating ear wound from a pneumatic nail gun. The nail passed through the stapes footplate, into the oval window, narrowly missing the facial nerve, and was lodged in the distal internal auditory canal. The patient underwent an extensive evaluation before the nail was safely removed in the operating room. This report describes the evaluation and management of penetrating wounds to the ear and temporal bone.

CASE REPORT

A 30-year-old man presented to the emergency department after being shot in the right ear with a nail from a pneumatic nail gun. He was afebrile, with stable vital signs, and complained of vertigo and decreased hearing on the right side. He had no significant medical or surgical history. On examination, the head of the nail was not visible and was buried beneath the skin anterior to the right external auditory canal. There was copious leakage of cerebrospinal fluid (CSF) from the right ear canal. Nystagmus was noted on left gaze. He was neurologically intact except for decreased hearing on the right as shown by tuning-fork examination.

A limited audiogram revealed a profound sensorineural hearing loss in the right ear. A high-resolution computed tomography scan of the temporal bone showed the nail beneath the skin of the anterior ear

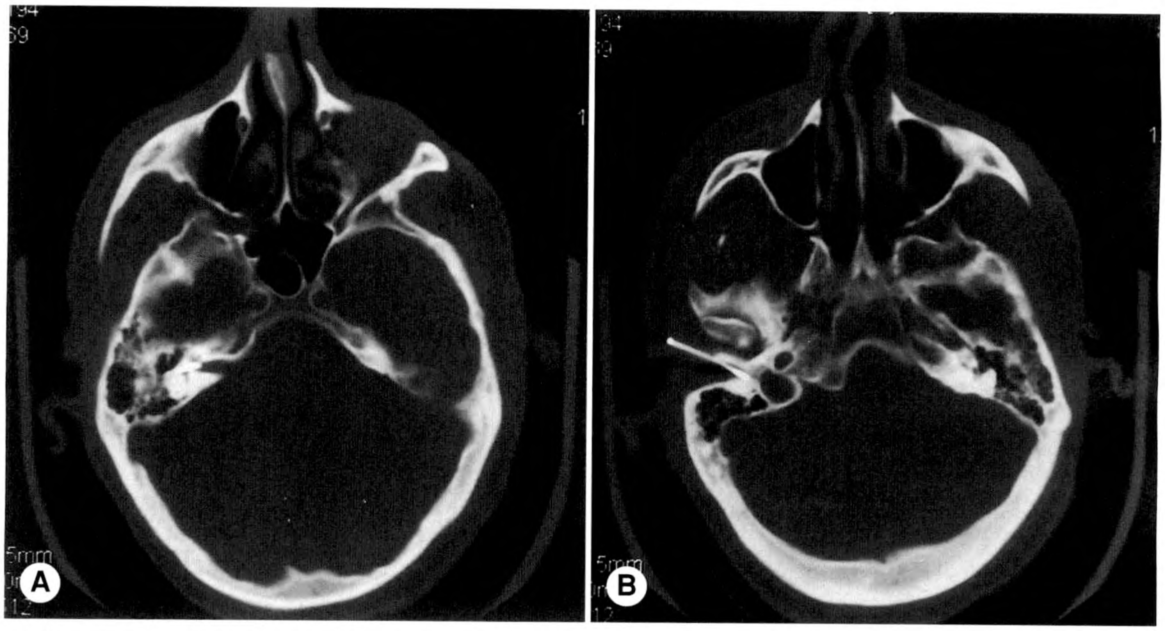

Fig 1. Axial high-resolution computed tomography scans of temporal bone. **A)** Distal portion of metallic foreign body (nail) can be seen penetrating oval window and reaching fundus of internal auditory canal. **B)** Metallic foreign body (nail) can be seen within external auditory canal, with medialmost portion seen in this image abutting region of jugular bulb.

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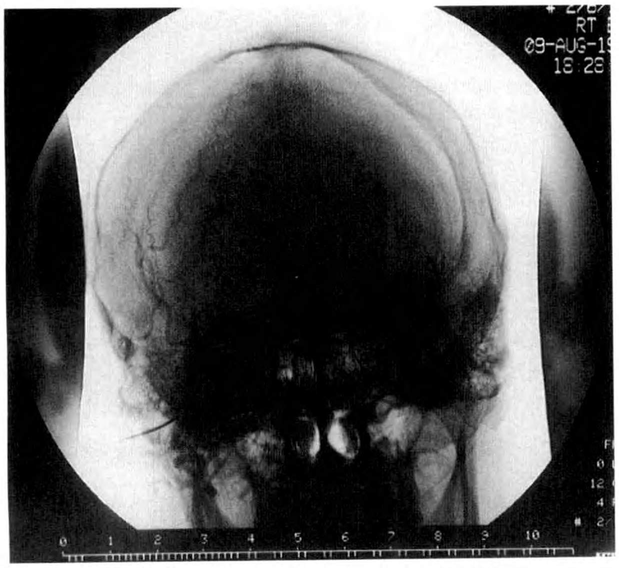

Fig 2. Coronal fluorograph demonstrates bent linear metallic foreign body (nail) in right temporal bone.

canal, extending into the middle ear, through the vestibule, and into the distal internal auditory canal (Fig 1A). The nail was 3 mm from the intrapetrous internal carotid artery and 1 mm from the jugular bulb (Fig 1B). An angiogram revealed no evidence of injury to the carotid artery, jugular vein, or sigmoid sinus. A scout film from the angiogram demonstrated the length of the foreign body in its entirety (Fig 2).

The patient was taken to the operating room, where he underwent a postauricular approach to a right middle ear exploration, removal of the foreign body, and obliteration of the middle ear cavity and eustachian tube for CSF control with abdominal fat and temporalis muscle grafts. After removal of the incus, the nail could be seen passing within 1 mm of the tympanic portion of the facial nerve and directly penetrating the stapes footplate and destroying the stapes (Fig 3). The stapes arch was free-floating in the middle ear and was removed. The facial nerve and chorda tympani were preserved. The carotid canal and jugular bulb were not violated. A spinal drain was placed.

The patient's recovery was remarkable for profound vertigo that lasted for several days, followed by a period of resolution. Of note, the patient complained of a stiff neck on postoperative day 1, yet was afebrile. A CSF analysis was obtained and was negative for evidence of infection, but the patient was treated with a 10-day course of intravenous ceftriaxone and nafcillin. The patient was discharged on postoperative day 7. Four months after surgery, the patient had a unilateral profound sensorineural hearing loss and mild residual dizziness.

DISCUSSION

Penetrating injuries of the ear may be caused by knives, nails, knitting needles, hairpins, cotton-tipped applicators, firearm projectiles, and a variety of other objects. Temporal bone injuries, like other forms of trauma, are more common in young men. One large series that examined temporal bone fractures due to all causes revealed that 76% of temporal bone fractures occurred in men, with 71% occurring between the ages of 11 and 40.[1] Penetrating injuries of the temporal bone may range from superficial canal lacerations to devastating injuries of the middle ear, facial nerve, carotid artery, jugular bulb, otic capsule structures, and intracranial contents.[2-6] The evaluation and management of potential injuries that should be considered in a penetrating wound of the ear and temporal bone are discussed below.

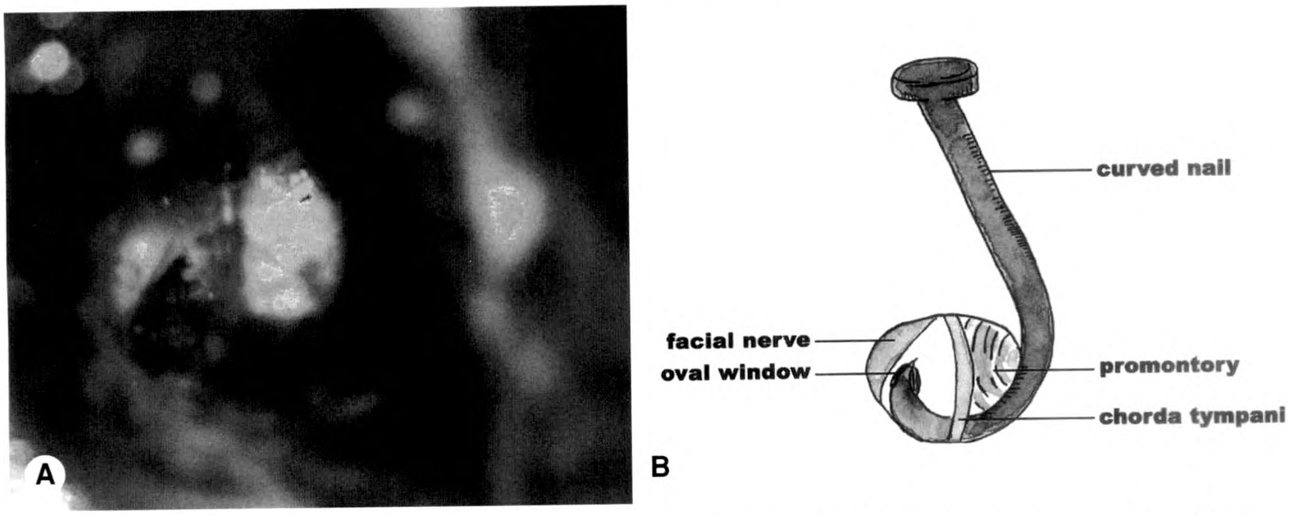

Fig 3. Operative findings. **A)** Intraoperative photograph of right ear canal and middle ear in surgical position reveals bent linear metallic foreign body (nail) passing down external auditory canal and coursing beneath chorda tympani nerve and through oval window. Note close proximity of facial nerve immediately superior to this region. **B)** Illustration corresponding to intraoperative photograph shown in A.

As with all trauma, treatment begins by securing the airway, stabilizing life-threatening wounds, and clearing the cervical spine of injury. When the patient is stable, attention is turned to obtaining a history from the patient or witnesses, such as the length of an object that might have been inserted or removed. Next, the physician examines the ear and the temporal bone and its associated structures. Any foreign body should be left in place until a complete examination is performed and a determination is made as to whether the patient should have the foreign body removed in a controlled setting. A physical examination should note any bleeding, ear canal trauma, tympanic membrane perforation, CSF leak, hearing loss, facial nerve injury, nystagmus, or neurologic deficit. A thorough evaluation is then performed to document fully the extent of injury.

Penetrating trauma is a rare cause of temporal bone fractures, accounting for only 3% of patients in a recent review of 820 such fractures.[1] Penetrating wounds are often divided into gunshot injuries and stab wounds. Tissue damage from gunshot wounds varies on the basis of the projectile mass and the square of the velocity of the projectile. The energy imparted from the projectile will cause tissue destruction in its path and often in a large area of neighboring tissue. Injuries caused by stab wounds are localized to the path of the offending object. Stab wounds may cause only superficial lacerations, or they may penetrate the depth of the temporal bone, resulting in considerable neurovascular trauma.[1] Computed tomography of the temporal bones is useful in assessing the relationship of the penetrating injury to the vascular structures of the temporal bone, facial nerve, and otic capsule.[7]

Vascular injury is suggested by the presence of bleeding or a bruit, as well as by the mechanism and location of the injury. When suspected, lesions such as arterial or venous transections, traumatic aneurysms, and arteriovenous and arterial–cavernous sinus fistulas should be excluded by an arteriogram with venous phases. Clinical examination alone is a poor predictor of vascular injury. There is no study of temporal bone vascular trauma that adequately addresses this issue, but in a study not involving the temporal bone, McCormick and Burch[8] reported that 42% of patients thought to have neck or peripheral arterial injuries on clinical evaluation had normal arteriograms, while 20% of patients thought to be free of arterial injuries had arterial damage identified on arteriography.

Vascular lesions may be treated by either surgery or embolization. Surgical repair or ligation of the vessel may be problematic because of the inaccessibility of vessels within the temporal bone and difficulty in obtaining proximal and distal control.[4] Briskly bleeding carotid injuries risk severe potential complications, and after initial control is obtained, these injuries may warrant consultation with an interventional neuroradiologist for management.

Hearing loss may be assessed initially with a 512- and 1,024-kHz tuning fork at the bedside, but a complete audiological evaluation should be performed as soon as the patient's condition is stable. It is important to differentiate sensorineural from conductive hearing losses. When sensorineural hearing loss is present, computed tomography scans may locate trauma to the otic capsule structures or even to the cochlear nerve in the internal auditory canal. A conductive loss may reflect the presence of debris or fluid in the middle ear, but may also represent a tympanic membrane perforation or ossicular discontinuity. If a conductive loss is noted and the patient is not vertiginous, an audiogram can be repeated in 3 months, thereby allowing for spontaneous resolution of many common causes of a conductive loss, such as effusion, hemotympanum, or tympanic membrane perforation. If ossicular discontinuity is present and reconstruction is necessary, surgery should be planned for no sooner than 3 months after injury. This delay will allow for resolution of edema or fluid within the middle ear.

Immediate facial paralysis in the setting of penetrating trauma implies transection of the facial nerve and requires early exploration.[7] At injury, a simple transection may be present, or a segment of nerve may be destroyed and an interposition graft may be necessary. Facial nerve weakness that progresses in the post-injury period may be secondary to edema, hematoma, or contusion and should be followed with serial electroneuronography if the patient reaches total paralysis. If a greater than 90% degeneration relative to the contralateral side is noted on electroneuronography within 14 days of injury, then a nerve exploration may be justified.[9]

Cerebrospinal fluid leaks caused by dural lacerations will often heal spontaneously with bed rest and head elevation. When conservative measures fail, CSF drainage via a lumbar drain may be necessary for as long as 4 days. Surgical repair by a transmastoid or craniotomy approach requires tissue for grafting of the defect. The use of prophylactic antibiotics remains controversial in the setting of a CSF leak.[10,11] In the patient reported in this study, ceftriaxone and nafcillin were started empirically after the patient developed a fever and nuchal rigidity in the immediate postoperative period.

After complete evaluation of the patient and the path of the stab wound, attention is turned toward removing the foreign body. Small, deeply embedded objects such as bullet fragments or shrapnel may be clinically followed if no sign of indolent infection is present. Larger objects that penetrate deeply and are not well visualized should be removed in the operating room. On the basis of the thorough preoperative evaluation described above, the surgeon should be prepared to manage vascular injury, ossicular damage, facial nerve injury, and CSF leak at that time.

The patient presented in this report had an unusual mechanism of penetrating temporal bone trauma. The evaluation and treatment of his wound were based on the principles of stabilizing the patient and systematically evaluating key anatomic structures that were at risk. This patient was particularly fortunate that the penetrating injury did not involve the facial nerve, carotid artery, or jugular bulb. Control of CSF otorrhea was successful, and the prognosis for further vestibular adaptation is excellent.

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LETTERS TO THE EDITOR

To the Editor:

I read with interest the article by Talmon et al published recently (Talmon Y, Samet A, Gilbey P. Total inferior turbinectomy: operative results and technique. Ann Otol Rhinol Laryngol 2000;109:1117-9). While the surgical technique they describe is laudable and the complications they report were minimal, their conclusions could be misleading. I make the following observations.

1. Only 102 of the 375 patients in the study had turbinate surgery alone. This represents only 28.6% of the patients they studied. The other patients had some form of nasal surgery (septoplasty, nasal polypectomy) that most likely accounted for the improvement in symptoms. It would have been more appropriate to analyze their results in patients who had turbinate surgery alone, as this was the procedure being studied.

2. Symptoms are known to recur in a certain percentage of patients even after total turbinectomy.[1] The patients in this retrospective study were followed up for 6 years, but the authors, unfortunately, did not describe the responses of their patients after 6 years. While it is appreciated that most recurring symptoms would be within the first 2 years, the long-term benefits of inferior turbinectomy surgery would be worthwhile to add to the literature on this subject. In the meantime, we still have to warn our patients that the benefits of surgery will not be long-standing in a percentage of patients.

3. While it is clear that the authors selected their patients for this procedure, it is unclear what the selection criteria were. They only state that all patients suffered from chronic nasal obstruction and failed to respond to local and systemic treatment. This diagnosis is unfortunately vague. The point is raised because it is important to note that inferior turbinectomy is usually an adjunct to medical treatment for nasal obstruction. The authors report that none of their patients required further medical treatment. It must be stressed, however, that if the underlying cause of the turbinate hypertrophy is not addressed, recurrent obstruction can and probably will occur in the long term.

Once again, I reiterate that the technique described is commendable and should encourage surgeons to perform this procedure where indicated, as it has its benefits and the feared side effects can be minimized. The benefits of the procedure, however, need to be cautiously interpreted.

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REFERENCE

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To the Editor:

We thank Dr Olarinde for his observations and are grateful for the opportunity to comment on them.

The article is about total inferior turbinectomy. The usual patient has more than one cause for his or her complaints and usually requires a combined procedure. Therefore, it is more appropriate to analyze reality rather than a theoretical model. Second, no operation has a 100% success rate, and inferior turbinectomy is no exception. Patients should be warned. Unsuccessful intervention is usually due to reasons other than the excised inferior turbinates: inferior turbinates do not regenerate after total trimming. I see patients who underwent total turbinectomy 20 years ago and still enjoy the results of the operation. A failure of the procedure might be due to reasons such as an abundant soft palate or lack of a nasal valve. Recurrent symptoms are due to conditions such as nasal polyps or hypertrophy of the medial turbinates.

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Nahariya, Israel

To the Editor:

I read the paper by Talmon et al (Talmon Y, Samel A, Gilbey P. Total inferior turbinectomy: operative results and technique. Ann Otol Rhinol Laryngol 2000;109:1117-9) with great interest. We ourselves perform total (or subtotal) inferior turbinectomies routinely in our department. Whenever we discuss this procedure with our American colleagues, they condemn it because of the risks of ozena or "empty nose syndrome." It seems that they often regard us almost as "criminals" when we mention inferior turbinectomy!

During the last 15 years, 1,156 patients underwent total or subtotal inferior turbinectomies (alone or in conjunction with septoplasty) at Hadassah University Hospital. We hope to publish our exact results in the near future, but in the meantime, I can definitely say that we have had a very good experience with this procedure. Almost all the patients are very satisfied with the relief from their nasal symptoms. The encouraging results have even caused us at times to refrain from performing septoplasty in cases with a mild septal deviation and turbinate hypertrophy that is unresponsive to medical treatment. Our experience has led us to believe that these cases can improve solely with inferior turbinectomy, and that septoplasty may not be required at all.

The most serious complication encountered by us was massive epistaxis requiring anterior and posterior nasal packing. There were only a few cases of atrophic rhinitis, and no cases of ozena or of empty nose syndrome. Some patients developed crusting, but it was mostly temporary and was relieved by nasal irrigation and application of lubricating gel.

The results of our very large series are therefore in line with the results published by Talmon et al. Hence, I join in their conclusion that total inferior turbinectomy is effective and relatively safe, at least in our Israeli climate.

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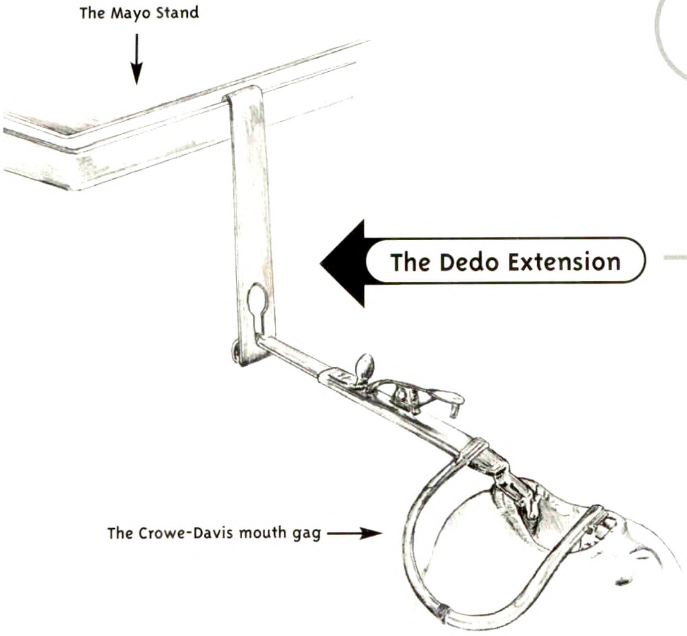

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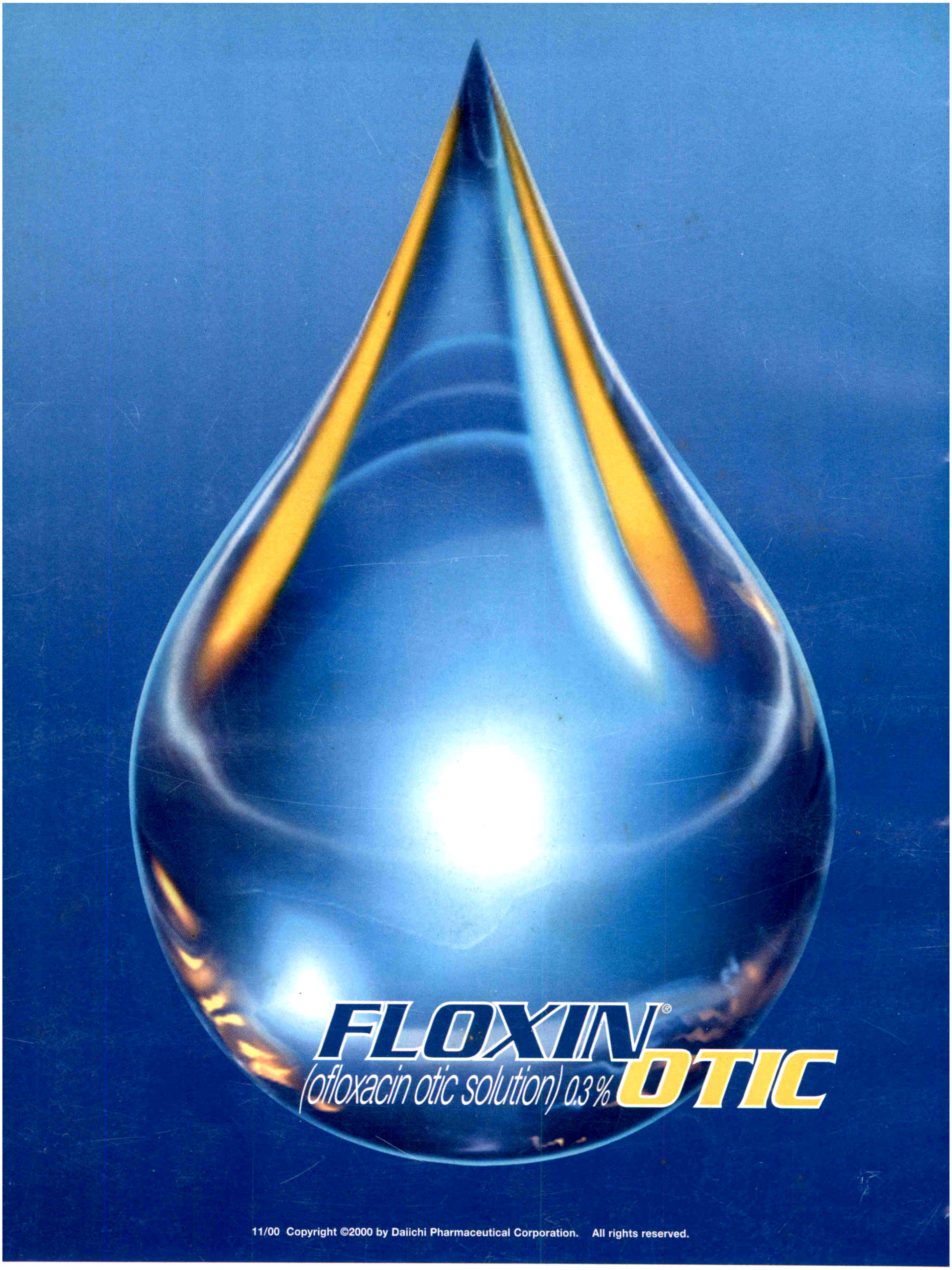
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SUPPLEMENT 187 — JULY 2001
Volume 110, Number 7, Part 2

ACTIVITY OF LARYNGEAL AFFERENTS DURING RESPIRATION, EVOKED VOCALIZATION, AND PARALYSIS

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II. EFFECT OF RECURRENT LARYNGEAL NERVE PARALYSIS ON SUPERIOR LARYNGEAL NERVE AFFERENTS DURING EVOKED VOCALIZATION

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Ann Otol Rhinol Laryngol 110:2001

I. INTERNAL SUPERIOR LARYNGEAL NERVE AFFERENT ACTIVITY DURING RESPIRATION AND EVOKED VOCALIZATION IN CATS

KEITH F. CLARK, MD, PHD JAY P. FARBER, PHD

OKLAHOMA CITY, OKLAHOMA

The purpose of this project was to identify and categorize patterns of activity of the internal branch of the superior laryngeal nerve during vocalization evoked by midbrain stimulation in cats anesthetized with α-chloralose. Unit activity was isolated from the cut distal end of the internal branch of the superior laryngeal nerve by means of floating bipolar electrodes that retained their contact with nerve fibers despite movement due to vocalization. The phases of respiration and vocalization were determined with a diaphragm electromyogram, a photoglottogram, and a microphone recording. Five groups of discrete afferent activities were defined according to relationships between the spike activity and the phases of vocalization. Group 1 cell activity peaked just before phonation, during expiratory airflow ($n = 26$). Group 2 cells ($n = 19$) followed a vocal fold vibratory pattern during phonation. Group 3 cell activity ($n = 6$) peaked during phonation, but did not follow vocal fold vibration. Group 4 cells ($n = 3$) were active during inspiration between phonations. Group 5 cells ($n = 4$) showed both inspiratory and expiratory modulation. These results indicate that laryngeal afferent activity responds to phonation-specific events during vocalization. This stereotyped afferent information might be used by the central nervous system to modulate vocalization.

KEY WORDS — larynx, mechanoreceptors, superior laryngeal nerve, vocalization.

INTRODUCTION

The larynx is richly innervated through the superior laryngeal nerve (SLN) by mechanosensitive afferents. Many laryngeal receptors are capable of following vibration, light touch, airflow, applied pressure indentations, transmural pressure, displacement of laryngeal structures, and contraction of the laryngeal muscles.[1-6] These types of stimuli are expected to be present during vocalization; however, the activity of laryngeal receptors during vocalization has not been systematically evaluated. Furthermore, many of the data describing laryngeal receptor behavior come from experiments that required laryngofissure to gain exposure to laryngeal structures while the animals were paralyzed and mechanically ventilated. A controversial hypothesis was proposed by Adzaku and Wyke[7] and Wyke[8] to suggest that laryngeal prephonatory tuning and motor control during vocalization depend on afferent discharges from a "triad of receptor systems" including mucosal, muscle, and joint mechanoreceptors. However, this view was not supported by other investigators.[9,10] Instead, the idea of central patterning of vocalization was proposed,[11] as well as control of vocalization via pulmonary receptors.[9]

Experiments in which sensory activity from the larynx was reduced by anesthesia *do* support the idea of a functional role for a proprioceptive system during vocalization. In terms of general, nontaxing phonatory tasks, oral, pharyngeal, and laryngeal topical anesthesia in human volunteers has shown only relatively minor effects.[12,13] During topical anesthesia, patients could not produce vocal fry,[12] and in high-pitched loud phonation, glottal resistance and subglottic pressure were higher after a block of the internal branch of the SLN (iSLN) in comparison to nonanesthetized controls.[14] A study of the effects of topical anesthesia and iSLN section in cats during electrically evoked vocalization reported increased hoarseness (decreased harmonics-to-noise ratio) and a decrease in fundamental frequency.[15] Afferent information may play a *greater* role during the initiation phase of phonation, before auditory feedback control can occur.[14] Although the methodology of pitch perturbation analysis has been challenged,[16] when the initial portion of phonation is examined in phonatory tasks in which extreme speed and/or accuracy of high-frequency control is required, anesthesia of the laryngeal mechanoreceptors appears to be detrimental.[17] Marked perturbations of pitch were noted during the first 15 cycles of phonation after anesthesia,[18] and iSLN block and mucosal anesthesia in volunteers also showed that the fine control mechanism of early phonation was adversely affected.[13,17,19]

From the Departments of Otorhinolaryngology (Clark) and Physiology (Farber), University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma. Supported by Presbyterian Health Foundation (PHF #162 and PHF #771); the American Laryngological Association; and the National Institute on Deafness and Other Communication Disorders (NIDCD) of the National Institutes of Health (R03-DC-01181-01A1). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Oklahoma Health Sciences Center.

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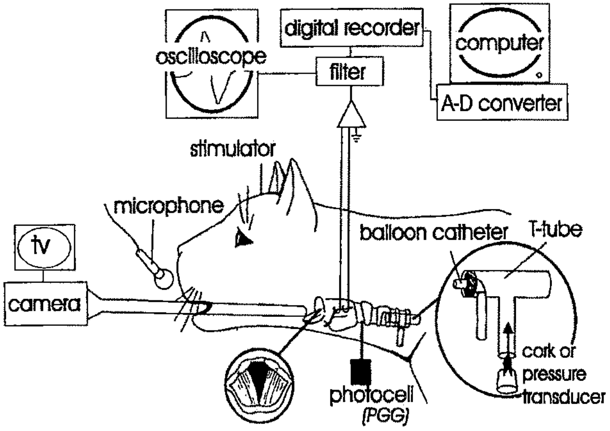

Fig 1. Experimental setup. See Materials and Methods for description. PGG — photoglottogram.

The division of laryngeal receptors into pressure, flow, and drive receptors by Mathew et al[20] has proven very useful for evaluating receptor characteristics, as well as reflex responses elicited from the larynx. However, the experimental manipulations required to classify receptors in this manner are very difficult to perform in a vocalizing animal. In addition, this classification system relies on collapsing negative airway pressure, which is not seen during vocalization. A complementary classification system examines slow versus rapid adaptation and the presence of spontaneous activity in deep versus superficial receptors.[1,2,4,6] However, it would be difficult to clearly segregate fibers into a location or adaptation category in an intact preparation.

To further understand the role of laryngeal receptors in control of vocalization, we must determine the effects of vocalization on afferent activity. In this study, vocalization was elicited by electrical stimulation in the region of the caudal midbrain periaqueductal gray (PAG) in anesthetized cats. "Single-unit" nerve fiber activity in the iSLN was studied, since this nerve provides the bulk of laryngeal afferent innervation. The kinds of vocalization-induced events observed among tested single units were measured and categorized with a new system based on vocalization behaviors. The results showed several patterns of vocalization-dependent responses. These could provide an important afferent limb for feedback control during phonation.

MATERIALS AND METHODS

GENERAL

All procedures were performed under the guidelines and approval of the University of Oklahoma Institutional Animal Care and Use Committee in compliance with "The Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 85-23, revised 1985). Repetitive vocalizations were produced in α-chloralose–anesthetized cats by stereotactically guided electrical stimulation of the midbrain PAG (Fig 1). Single-unit recordings were made from isolated fibers of the distal stump of the sectioned iSLN during vocalizations. Simultaneous recordings were made of the voice, photoglottograms (PGGs), diaphragm and cricothyroid (CT) electromyograms (EMGs), and the tracheal pressure.

ANESTHESIA

The cats were first tranquilized with intramuscular ketamine hydrochloride (10 mg/kg). Then, 1% Xylocaine with 1:100,000 epinephrine was injected into the groin, and femoral vein and artery catheters were placed for drug administration and blood pressure monitoring, respectively. An initial dose of α-chloralose (average, 35 mg/kg) was given intravenously. Additional intravenous doses of α-chloralose (average, 5 mg/kg) were given over a 1.5- to 3-hour time period at 30-minute intervals until the limb withdrawal reflex to paw pinch became consistently absent, blood pressure stability occurred, and the pupils remained constricted. Care was taken to provide complete anesthesia before surgical manipulation. Additional anesthetic was given during the experiment as necessary to maintain anesthesia as measured by absence of limb withdrawal and pupillary dilatation with paw pinch. Atropine sulfate (0.06 to 0.16 mg; 3 of 21 cats) and pentobarbital sodium (0.03 to 0.13 mg/kg; 3 of 21 cats) were given to control secretions and decrease chloralose-induced hyperreflexia. The blood pressure was recorded with a pressure transducer connected to the femoral artery catheter. The rectal temperature was controlled (37°C ± 1°C) by an automatic heating blanket. At the end of the experiment, the animals were euthanized by a pentobarbital sodium overdose (>200 mg/kg).

VOCALIZATION

The anesthetized cats were placed in a head holder. A craniotomy was prepared to position a stimulating electrode at stereotaxic coordinates of zero in the anterior-posterior plane and 1.5 mm lateral in the medial-lateral plane. Then, the electrode was positioned in the dorsal-ventral plane at 0.5-mm increments to the range of 15 mm above the interaural plane until the strongest phonation was produced with the least stimulating current. The expected electrode placement based on the stereotaxic coordinates was in the general region of the caudal midbrain PAG. Stimulating parameters of 1 to 3 mA, 200-μs pulse duration, at 100 Hz were used to produce vocalizations.

NERVE DISSECTION

A neck incision was made, and the skin was used to contain a pool of warm mineral oil (39°C). The iSLN was transected and the neural sheath removed from the cut end of the portion arising from the larynx. Slips of the nerve were teased apart into fibers, which were placed over platinum, bipolar hook electrodes immersed in mineral oil. Fixation of the electrode complex to the laryngeal skeleton with cyanoacrylate cement and epoxy allowed the electrodes to move with the nerve. This design prevented the nerve from being pulled off the electrode complex when the animal swallowed, coughed, or vocalized.

PERIPHERAL NERVE RECORDING

Action potentials from iSLN single units were high-pass fourth-order filtered at 20 Hz, amplified with a preamplifier (model DAM-5A, World Precision Instruments, Sarasota, Fla), digitally recorded, and monitored on an oscilloscope and an audio monitor.

RECORDING PHONATION

Acoustic data were acquired via a custom-built amplifier and microphone (model UA0196, Bruel and Kjaer, Copenhagen, Denmark) placed 15 cm from the mouth. This gave an indication of the onset and offset of phonation and the relative intensity. The output from the microphone was passed to the oscilloscope and digitally recorded.

MONITORING VOCAL FOLD MOVEMENTS

The tip of a flexible fiberoptic endoscope (Olympus EnFP-2, Hitschfel Instruments Inc, St Louis, Mo) with an attached video camera was passed transorally and suspended inside the hypopharynx, above the laryngeal inlet. The endoscope illuminated the larynx from above and served as a light source for the PGGs. Photoglottography is a technique that uses a photoelectric transducer to describe time-varying changes in glottal area.[21] Light from the endoscope aimed through the glottis was transduced by a photosensor (OSD-60, Centronics Inc, Newbury Park, Calif), with a custom-built amplifier, placed on the neck just below the cricoid cartilage. The PGG signals were monitored on an oscilloscope to assess the adequacy of the glottographic signal. The PGG data were used to indicate vocal fold movements and provide vibration frequency and timing information for 10 single units.

ELECTROMYOGRAPHY

The EMG activity of the diaphragm and CT muscles was monitored with bipolar electrodes placed into the respective muscles. The electrodes were prepared by removing 2 to 3 mm of insulation from a 30-gauge stainless steel wire and passing the bared end of the wire just through a 25-gauge hypodermic needle. The bared portion of the wire was bent approximately 140° to create a hook that was then passed into the target muscle and remained anchored after the needle was removed. The costal diaphragm muscle was exposed through a midline skin incision over the xiphoid process. The 2 hook-wire needle electrodes were passed just behind the xiphoid process, parallel to the plane of the diaphragm muscles, under direct vision. This placement minimized contamination of the EMG by intercostal muscle activity and was confirmed by the timing of the diaphragm EMG. The electrodes were placed into the CT muscle under direct vision. Each EMG was amplified (model 7DAG, Grass, West Warwick, RI), viewed on the oscilloscope, and digitally recorded.

TRACHEAL T-TUBE AND TRACHEAL PRESSURE

A silicone T-tube was placed through a tracheotomy into the trachea. A pressure transducer (model P23AA, Stratham, Electromedics, Englewood, Colo) with a differential amplifier (Grass model 7DAG) was attached to the external limb of the T-tube via rigid plastic tubing to allow recording of tracheal pressure. In addition, a probe or catheter was passed through the T-tube to touch glottic and subglottic sites or to apply topical anesthetic.

CLASSIFICATION OF RECEPTORS

Approaches described in the literature for locating receptors or characterizing adaptation rates were considered inappropriate for this study, since most of those manipulations require chemical paralysis or laryngofissure, which would eliminate the possibility of phonation. It was also impractical to combine our stereotaxic device and fiberoptic scope with a mask to perform the protocol of Sant'Ambrogio et al.[5] Although the experimental conditions described in the literature are very different from ours, we first tried to stratify the characteristics of our receptor responses during spontaneous respiration into silent (<10 Hz) and tonically active (10 to 80 Hz) categories.[1,2] Then, all single-unit responses were classified as to how the action potential rate (spike rate) changed during spontaneous respiration (control sequence) and during PAG stimulation (vocalization sequence). The control and vocalization sequences were divided into inspiratory phases called INSP-CONT and INSP-VOC, respectively. The expiratory phase of the control sequence was called EXP-CONT. The expiratory phase of vocalization was further divided into prephonatory and phonatory phases (Table 1 and Fig 2). Visual inspection of the instantaneous mean frequency (ImeanFr, channel 1) tracing over

TABLE 1. CONTROL AND VOCALIZATION PHASES

Phase	*Abbreviation*
Spontaneous respiration (control)	
Inspiratory control	INSP-CONT
Expiratory control	EXP-CONT
PAG stimulation (vocalization)	
Inspiratory, PAG stimulation	INSP-VOC
Expiratory prephonatory	EXP-VOC
Expiratory phonatory (vocalization)	PHON

Control respiration and periaqueductal gray (PAG) stimulation sequences are divided into phases and labeled as shown.

time was used to determine whether the spike rate changed during each phase. The ImeanFr is the reciprocal of the time between 2 spikes. The ImeanFr tracing displayed spike-to-spike changes in firing rate.

A limited number of single units were further characterized by their responses to the following manipulations: 1) passing a probe through the T-tube and the mouth to touch supraglottic, glottic, and subglottic sites (the anatomic site was observed with the video equipment) and 2) placing topical anesthetic (1% Xylocaine) to categorize a limited number of single units into superficial and deep locations. When a single unit responded to probing and displacement of the epiglottis, the recording was repeated after removal of the fiberoptic scope. This maneuver was done to ensure that an increase in spike rate was not due to contact of the epiglottis with the fiberoptic scope.

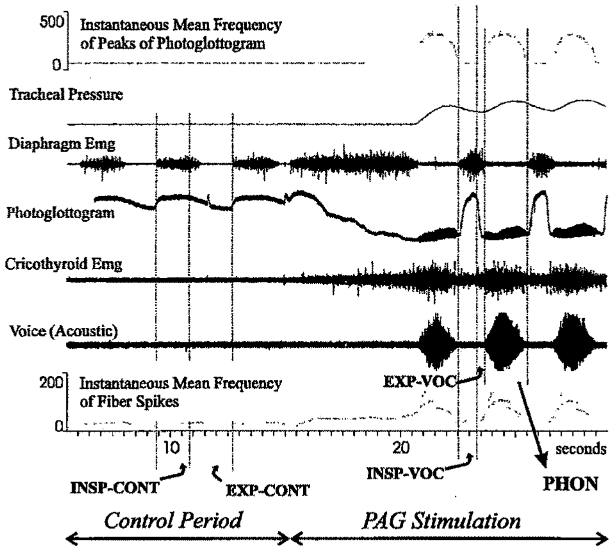

Fig 2. Phases of respiration and vocalization. Record of control and periaqueductal gray (PAG) stimulation sequence made while recording tracheal pressure, diaphragm electromyogram, PGG, cricothyroid electromyogram, and voice. Computer is used to identify instantaneous mean frequency (ImeanFr) of peaks of PGG tracing on top channel and ImeanFr of fiber action potentials on bottom channel. Phases described in Table 1 are labeled for reference. Artifactual offset seen during control period on ImeanFr of peaks of PGG channel is due to ambient 60-cycle noise. (See also Fig 4.)

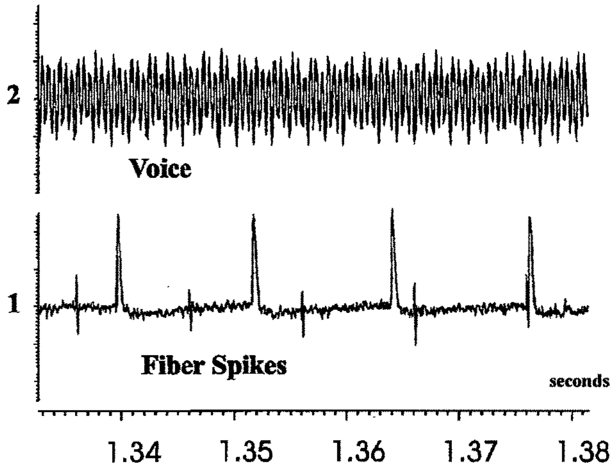

Fig 3. Raw record. Microphone recording of phonation is displayed on channel 2, with simultaneous action potential activity for single fiber on channel 1. Spike recognition algorithm utilizes spike amplitude and shape to ensure that action potentials from only 1 fiber are being analyzed. In addition, artifact from electrical stimulation of PAG is also recognized and eliminated from analysis.

DATA ANALYSIS

General. The data were analyzed off-line by computer. Inspiration was defined by eye as starting at the onset of diaphragm EMG activity and slight vocal fold abduction (seen on the PGG) and ending as the diaphragm EMG activity began to fall from its maximum level. Expiration was defined as starting with the decline of the diaphragm EMG activity, as well as vocal fold adduction (seen on the PGG), and ended as the next diaphragm EMG activity began. Single-unit action potentials (spikes; Fig 3) and voice acoustic analog signals were sampled at 22 kHz, while the EMG, PGG, and tracheal pressure analog signals were sampled at 11 kHz. The digitizer (Neurocorder, Neurodata Inc, New York, NY) saved the information onto a videocassette recorder that later played the signals to a computer-based waveform analysis system (1401-plus, Cambridge Electronic Design, Cambridge, England).

All spike recordings were initially inspected by eye. Spikes from individual afferent single units were selected, in some instances by voltage level, but more often by use of a computer software spike recognition algorithm (Spike 2, version 4.0, Cambridge Electronic Design) that utilized spike amplitude and shape to ensure that only a single axon (single unit), rather than a population of single units (multiunits), was selected (Fig 4). The software selected spikes that crossed a waveform signal trigger level to create templates based on spike shape. Spikes were defined as 100 points per spike, and template matching required 60% of the data points to lie within the template for a single spike. These spike occurrences were further converted to electronic events with an associated

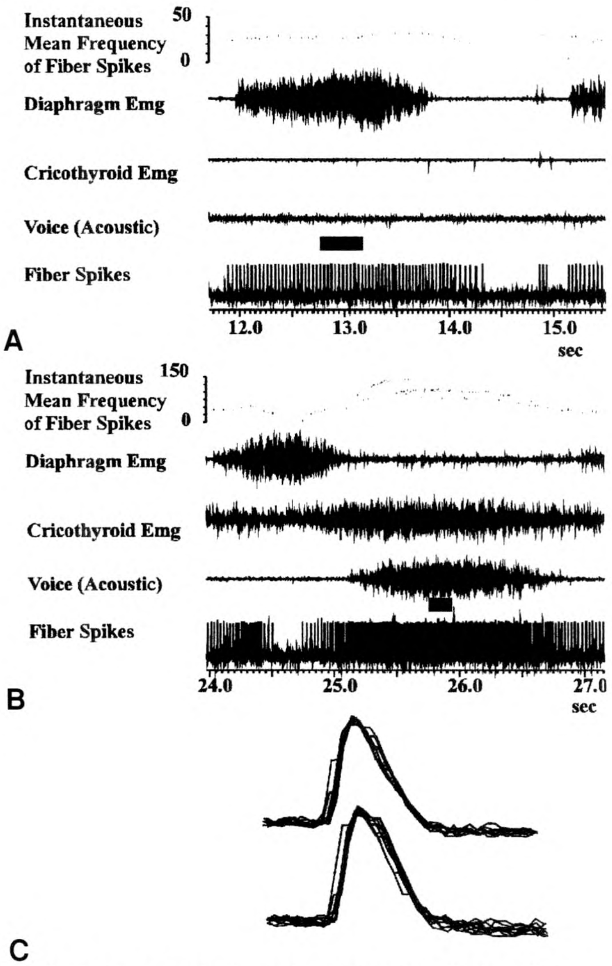

Fig 4. Shape analysis of phase-changing single-unit fiber. **A)** Control inspiration. **B)** Phonation. Fiber depicted in Fig 2 was classified as group 3 single-unit fiber with peak activity during phonation and not frequency-following. If classified during spontaneous respiration, it would be considered to be inspiratory-modulated. Examination of PAG stimulation sequence reveals that activity has switched, with pauses during inspiration and maximum activity during phonation (expiration). In order to eliminate possibility that second fiber became active, action potentials from raw record were superimposed. This analysis reveals that action potentials maintain amplitude and shape during both phases. Black bars over fiber spike channels show where action potentials were taken.

ImeanFr tracing plotted against time. This plot was also inspected to confirm the presence of smooth transitions of frequency associated with single-unit activity. The computer then converted the PGG waveform to a series of electronic events, which represent the points in time at which the vocal folds were fully opened. These PGG peaks were also associated with an ImeanFr value and plotted against time. The PGG peaks served as markers between vocal fold vibration cycles and as the trigger event for cross-correlation with neuronal spike activity.

Two types of cross-correlation graphs were then created from the PGG peak and spike events: a phase histogram and a cross-correllogram. The phase histogram was used to generate statistical data, from a short segment of phonation, by which comparisons could be made between single units. The phase histogram was generated for 100 spike events in the central portion of a single phonation. Every PGG cycle was divided into 10 equal parts (bins), and the number of spikes occurring during each bin was stored in an array. This array provided data for calculation of the mean, the standard deviation, and the η^2 value as described below. The cross-correllogram provided a comparison of single-unit firing rate and synchrony with vocal fold vibration over an entire phonation. The cross-correllogram was created by finding the occurrence of spikes over a 30-ms observation window initiated with a spike; the 30-ms observation window was then repeatedly shifted to the next spike occurrence, and the incidence of spikes during the interval was recorded. The cross-correllogram identified activity that had a constant firing rate over time. It was affected more by changes in vibration frequency than was the phase histogram. If there was variation in the pitch (frequency) of the phonation during the interval being examined, then the cross-correllogram appeared to show a poorer correlation. The phase histogram is better suited for an activity, like phonation, that has a changing frequency, since it subdivides the period of each cycle.

The ImeanFr of the PGG peaks and the ImeanFr of the single-unit spikes were directly compared to see whether action potentials followed vocal fold vibration and to calculate a "rate of frequency following." This could be at the frequency of vibration or at some harmonic. The rate-of-following histogram indicated the number of times the activity of a single unit followed vocal fold vibration at each of the first 10 harmonics.

Quantification of Vibration-Following Activity: η^2 Value. The essential feature of vibration-following activity is that it occurs only during phonation, and that it is in phase with some component of the vocal fold vibration cycle. The statistical methods summarized below were used by Orem and Dick[22] to analyze the size of the respiratory component in activity of medullary neurons. These methods were applied to characterize the size of the vocal fold vibration–following component in iSLN neurons activated during phonation. The neuronal activity was arranged in a matrix in which the columns were fractions of the vibration cycle and the rows were vibration cycles. This matrix was analyzed with a 1-way analysis of variance (ANOVA) to determine whether the neuronal activity contained a vibration-following component. The ANOVA was a subjects-by-treatments (repeated-measures) design with deciles of the vocal fold vibration cycle as the "treatments" variable and cycles ($N = 100$) as the "subjects" variable. The vibration cycles associated with 100 consecutive iSLN spikes were selected from the flat central portion of the single phonation judged to have the least cycle-to-cycle variation on the ImeanFr tracing of the PGG peaks. When the vibratory frequency was low, it was not always possible to obtain 100 vibration cycles from 1 phonation. In this case, four 25-cycle blocks were accumulated from the mid-portions of several phonations.

The η^2 value was used to quantify the *degree* of vibration-following detected by the following ANOVA.[22]

$$(1) \qquad \eta^2 = \sigma m^2/(\sigma m^2 + \sigma^2)$$

The η^2 value in equation 1 is the proportion of the total variance of the neuronal activity over a series of vibration cycles that is made up of the variance across fractions of the vibration cycle. The term σ^2 is the variance across vibration cycles within the individual fractions of the cycle. The term σm^2 represents the variance of activity across fractions of the vibration cycle. The η^2 value can also be estimated from the ANOVA in equation 2.[22]

$$(2) \qquad \eta^2 = dfb(F)/(dfb(F) + dfw)$$

In equation 2, F is the F ratio, dfb is the degrees of freedom between groups (or degrees of freedom for the sum of squares for subjects), and dfw is the degrees of freedom within groups (or degrees of freedom for the error-term sum of squares). The η^2 value is an estimate of the magnitude of an experimental effect and can range from 0.0 to 1.0, indicating that none to all of the variability in a data set is caused by the treatment variable. For example, an η^2 value of 0.8 would indicate that 80% of the variability of the activity of the neuron was due to the effects of the vocal fold vibration cycle.

RESULTS

Data analysis was possible in 21 of the 54 cats used in this study. Evoked phonation could not be obtained in 15 animals. Technical problems such as deterioration of the preparation, poor phonation, difficulty in teasing apart the iSLN nerve slips, difficulty in maintaining contact with the electrode during vocalization, multiunit recordings, and excessive stimulus artifact prevented valid data acquisition from 18 cats.

CENTRALLY EVOKED VOCALIZATION

The midbrain PAG region was selected as the area most likely to evoke vocalization when electrically stimulated. Stimulation sites that yielded vocalizations were found along a narrow band centered on a line 1.5 mm lateral to stereotaxic zero that extended approximately 3 mm rostral and caudal. The best site chosen in each animal yielded clear, natural-sounding vocalizations with a minimum of accessory behaviors. The expected electrode placement based on stereotaxic coordinates was in the general region of the caudal midbrain PAG, consistent with Berman's stereotaxic atlas.[23] It is likely that PAG cells, as well as output pathways, were stimulated. Other investigators have reported coordinates that would have placed the electrode outside the PAG.[10,11]

STIMULATION PARAMETERS

Once the optimum site for stimulation was identified, the intensity, duration, and frequency of the electrical stimulus were varied. The most important parameter was stimulus intensity. The volume and clarity of the vocalizations were directly proportional to the stimulus intensity. Both low and high current levels evoked 2 to 5 cycles of phonation followed by inspiration. Lower current levels produced a whisper-like phonation that became more vocal as the current level was increased to the 1- to 3-mA range. Current levels above 3 mA were associated with an intense, distorted vocalization and accessory movements. Stimulus conditions were selected to produce a subjectively "good" vocalization, but vocalizations could not be reproduced identically among animals.

VOCAL FOLD VIBRATION

The majority of phonations produced had a vibration frequency of approximately 250 Hz. Some animals reached a vibration frequency of more than 600 Hz. Variation in the period of vocal fold vibration from cycle to cycle (jitter) was visible on the ImeanFr tracing of the PGG peaks.

SUPERIOR LARYNGEAL NERVE ACTIVITY

A total of 60 iSLN afferent nerve single units were isolated from 21 cats in this study. Categorization for silent versus tonic activity revealed 16 silent and 44 tonic response types. The patterns of single-unit spike rate activity occurring during PAG stimulation were more consistent and had much higher rates than those seen during control periods. The most commonly found afferents were activated during expiration ($n = 51$) with PAG stimulation. For this reason, the primary grouping was done by inspection of the PAG stimulation sequence. The average response rates of single units found during spontaneous respiration and PAG stimulation are presented in Table 2. Five groups of discrete activities were defined according to the patterns of spike ImeanFr seen during inspiration and expiration under conditions of stimulation of the PAG and/or its output pathway (Table 2).

TABLE 2. AFFERENT GROUPS

		Control Respiration		*PAG Stimulation Sequence*		
Group No.	*Group Description*	*Inspiratory INSP-CONT*	*Expiratory EXP-CONT*	*Inspiratory INSP-VOC*	*Expiratory EXP-VOC*	*Vocalization*
Expiratory						
1	Peak prephonation (n = 26)	*	*	8	108	74
2	Frequency-following (n = 19)	*	*	*	33	245
3	Non–frequency-following (n = 6)	35	36	42	48	76
Inspiratory						
4	Inspiratory (n = 3)	25	0	48	10	1
Mixed						
5	Inspiratory and expiratory (n = 4)	64	1	8	90	66
Miscellaneous						
6	Constant (n = 1)	10	10	10	10	10
	Independent (n = 1)	125	0	150	150	125

Primary grouping of activity of internal branch of superior laryngeal nerve was done by inspection of PAG stimulation sequence and revealed 6 groups. Data are average spike rates in hertz.

*Within these groups there were various patterns of activity; see Table 3 and text.

Group With Peak Before Phonation (Group 1). The spike rate of group 1 single units (n = 26) peaked during the prephonatory expiration phase (EXP-VOC) and generally remained constant until inspiration. Group 1 single units could reflect some aspect of laryngeal muscle activity, since their responses followed laryngeal adductor muscle contraction as evidenced by CT EMG. The spike rate peak seen in this group did not occur at the same time as the peak in CT EMG. Thus, group 1 receptors may respond to initial vocal process or arytenoid contact when the vocal folds close. Often, near-maximal activity would occur during PAG stimulation when the cat closed the glottis but did not produce enough effort to phonate. At these times, there was no vocal fold vibration, nor very much airflow, yet CT activity was prominent. There was no relationship between this closure and tracheal pressure or phonation. This group was further divided into 6 subgroups based on activity during control respiration (Table 3). Figure 5 depicts the activity of a typical group 1 (group 1b), expiratory-modulated single unit. This single unit responded at its maximum rate during early expiration before

TABLE 3. PRIMARY GROUPINGS BASED ON ACTIVITY DURING PAG STIMULATION

Group	*No. of Single Units*	*No. of Cats*	*INSP-CONT*	*EXP-CONT*	*INSP-VOC*	*EXP-VOC*
1a	5	4	0	0	0	111
1b	6	3	0	30	0	145
1c	4	2	25	25	0	73
1d	9	7	23	18	18	106
1e	2	1	21	0	15	43

Data are average spike rates in hertz. Subcategories are based on activity during control conditions. Group 1a — single units with no activity during either phase of control respiration or during INSP-VOC; group 1b — single units with no activity during either INSP-CONT or INSP-VOC but with low rate during EXP-CONT (Fig 6); group 1c — single units with constant low rates during control respiration but silent during INSP-VOC; group 1d — single units with moderate, fairly constant rates during control respiration and INSP-VOC; group 1e — 2 single units with activity during INSP-CONT and INSP-VOC.

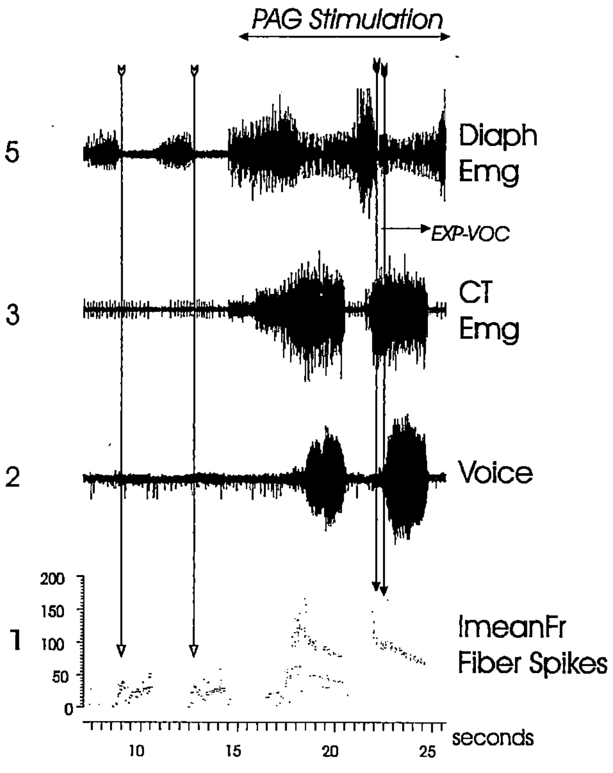

Fig 5. Prephonation-peak group 1 single unit. Control and PAG stimulation conditions are displayed for activity of typical expiratory-modulated single-unit fiber (group 1b). It responds with slight peak in early expiration during control respiration (white arrows), presumably at time of partial vocal fold adduction. Maximum rate during vocalization sequence occurs during early expiration as well. Prephonatory phase of expiration (EXP-VOC) is shown between black arrows. During phonation, spike rate gradually declines. There is very little or no activity during inspiration.

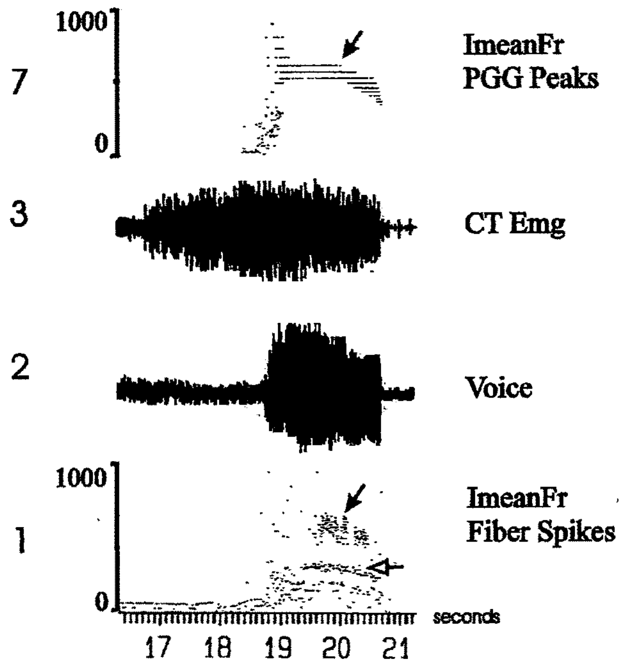

Fig 6. Frequency-following group 2 fiber during single phonation. One expiratory phase during PAG stimulation is depicted for vocal fold–following fiber (group 2). As is characteristic of group 2 fibers, activity correlates with onset and offset of phonation. This example depicts high spike rate (>600 Hz) that these neurons are capable of. Notice vertical spread of points on ImeanFr of PGG peaks (black arrow on channel 7) representing cycle-to-cycle variation in period (jitter) of approximately 100 Hz. This vibration following fiber's action potential rate shows similar jitter on ImeanFr spikes seen at black arrow on channel 1 (follows at 1:1). White arrow shows harmonic representing spikes that follow at half fundamental frequency (follows at 1:2) of PGG peaks. One cycle of vocal fold vibration occurs between each pair of PGG peaks.

each phonation (EXP-VOC). Of 4 single units tested with displacement of the epiglottis, 2 responded and 2 did not. One single unit from this group showed immediate loss of spike activity upon instillation of topical anesthetic.

Vibration Frequency–Following Group (Group 2). Group 2 single units (n = 19) had onset and maximum activity during phonation. These single units followed the vibration frequency of the vocal folds and tended to show low rates of activity or no activity during other phases. In contrast to the group 1 single units, the single units in group 2 did not respond during vocal fold closure, but became active during phonation. Two of the 5 group 2 single units tested responded to displacement of the epiglottis with a probe. One group 2 single unit responded to light touch of the vocal fold edge. The activity of 1 additional single unit was immediately quieted after application of topical anesthetic.

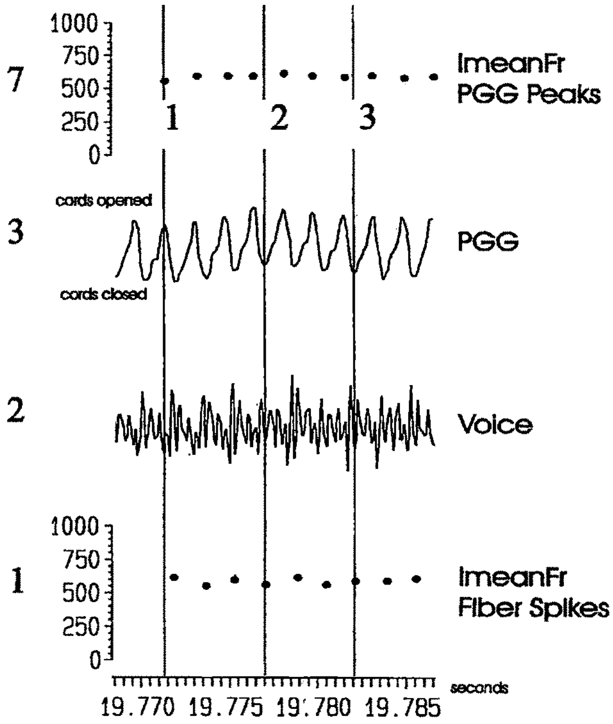

Fig 7. Identification of frequency-following single-unit fiber by inspection. Fifteen-millisecond portion of single phonation is shown from single-unit fiber depicted in Fig 6. Visual inspection of timing of PGG troughs (vocal fold closure) and fiber spikes reveals consistent relationship. Vertical line labeled 1 lines up with PGG peak. Each dot on channel 7, labeled "ImeanFr PGG Peaks," corresponds to vocal folds opened. Corresponding spike discharge is shown on channel 1. Spike occurrence is displaced by delay caused by fiber conduction to electrode. ImeanFr marking is lined up directly over peak of corresponding spike.

Eleven single units from 9 cats could be placed into 1 main group (group 2a) with low activity during control respiration and inspirations between phonations. The remaining single units were placed into a second group (group 2b; 8 single units from 4 cats) that showed a variety of activities during control conditions and during inspirations under PAG stimulation.

Figure 6 demonstrates the dramatic activation of a single unit to a rate of more than 650 Hz during the late portion of the first phonation. Figure 7 shows a 15-ms portion of a single phonation from the single unit depicted in Fig 6. Visual inspection of the timing of PGG troughs (vocal fold closure) and single-unit spikes reveals a definite relationship. The single unit seemed to fire at each vocal fold closure. This type of visual inspection was used to initially identify all of the group 2 afferents. Two other group 2 single units are shown in Figs 2 and 8. The single unit in Fig 8 almost exactly follows the vocal fold vibratory pattern as defined by the PGG waveform and follows at a rate of 1:1. Results of the cross-correlation analysis for vocal fold vibration–follow-

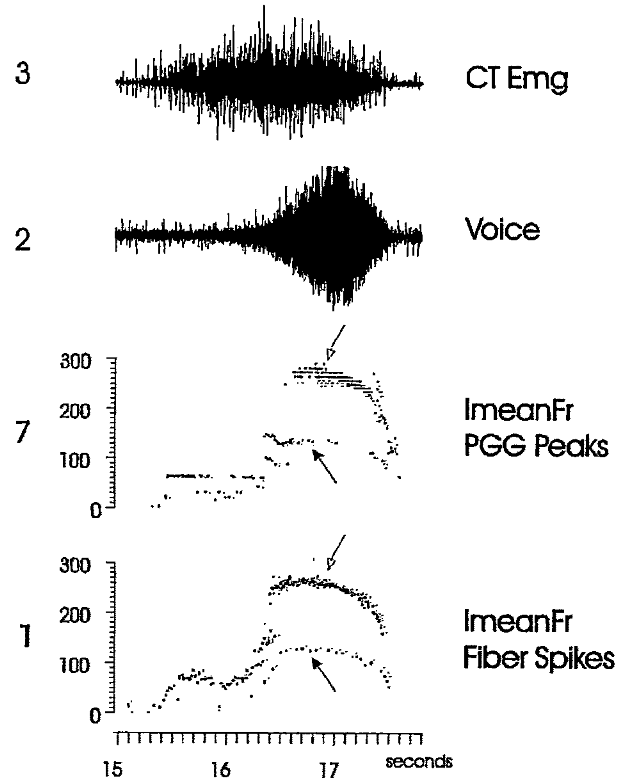

Fig 8. Frequency-following single-unit fiber during single phonation. This group 2 single-unit fiber almost exactly follows vocal fold vibratory pattern as defined by PGG waveform. Notice nearly identical shape of ImeanFr PGG peaks and ImeanFr fiber spike tracing, indicating that fiber is following vocal fold vibratory pattern at rate of 1:1. Fundamental frequency of voice is shown by white arrows. Black arrows show instances in which vocal fold intermittently vibrates at half of frequency shown by white arrows, according to PGG channel. In these instances, afferent appears to be accurately following this subtle perturbation.

TABLE 4. STATISTICS FROM GROUP 2, VIBRATION-FOLLOWING SINGLE UNITS

Single-Unit Name	*Frequency Follows*	*F Ratio*	*η^2 Value*
A	1:1	8	0.09
B	1:2	12	0.11
C	1:1	7	0.11
D	1:2	17	0.15
E	1:1	15	0.18
F	1:2	30	0.24
G	1:3	54	0.36
H	1:2	150	0.60
I	1:1	2,174	0.96
J	1:1	>5,000	1.00

Results of cross-correlation analysis for vocal fold vibration–following activity for 10 single units with adequate photoglottography data. "Frequency Follows" describes relationship between vocal fold vibratory rate and spike rate. F ratio and η^2 value are calculated as described in text in order to stratify single units by how well they follow vocal fold vibration. Better following is indicated by higher F ratio and higher η^2 value.

ing activity for the 10 single units with adequate PGG data are listed in Table 4. Most of these single units seemed to respond at the same time in the vibration cycle, although processing delays prevent an exact determination of what is physically happening to the folds when the spikes occur. Figure 9 shows the ANOVA F ratio, η^2 value, phase histogram, and cross-correlation graph for 2 examples of group 2 single units as compared with 1 example of a single unit from group 3, which does not follow a vocal fold vibratory pattern.

The vibration-following behavior seen in group 2 single units does not appear to be due to mechanical vibration of the nerve or electrode. The activity of 1 of these single units was immediately eliminated by application of topical anesthetic, during control and vocalization sequences; this finding suggests that receptor activation was responsible. We often saw an artifact that transformed the single unit recording channel into something very similar to the tracing seen on the microphone channel. This was considered to be mechanical and due to vibration of the electrode. It duplicated the sound of the phonation on the audio amplification equipment meant to display the sound of single-unit action potentials. When this occurred, the recording was useless, and the experiment was not successful. It was usually caused when the nerve was stretched when attached to the recording electrodes. When the nerve was long enough to be lax during phonation, we never saw this type of artifact.

Non–Peak Phonation Frequency–Following Group (Group 3). Group 3 single units (n = 6) had unique patterns of spike activity during control respiration and peak activity during phonation that did not follow vocal fold vibration (Figs 9C and 10). None of these single units was tested with topical anesthetic.

Inspiratory-Modulated Group (Group 4). Group 4 single units (n = 3) were active only during inspiration during both control and PAG stimulation conditions. Group 4, inspiratory-modulated single units may have been responding to vocal fold movements or posterior cricoarytenoid muscle contraction, rather than airflow. None of these single units was tested with topical anesthetic.

Mixed Group (Group 5). Group 5 single units (n = 4) showed brief peaks before, during, and after phonation, but no activity during control sequences. Three single units in group 5 responded when the posterior glottis was touched with a probe. None of these single units was tested with topical anesthetic.

Miscellaneous. On the basis of the relatively large number of afferent groups found as compared with

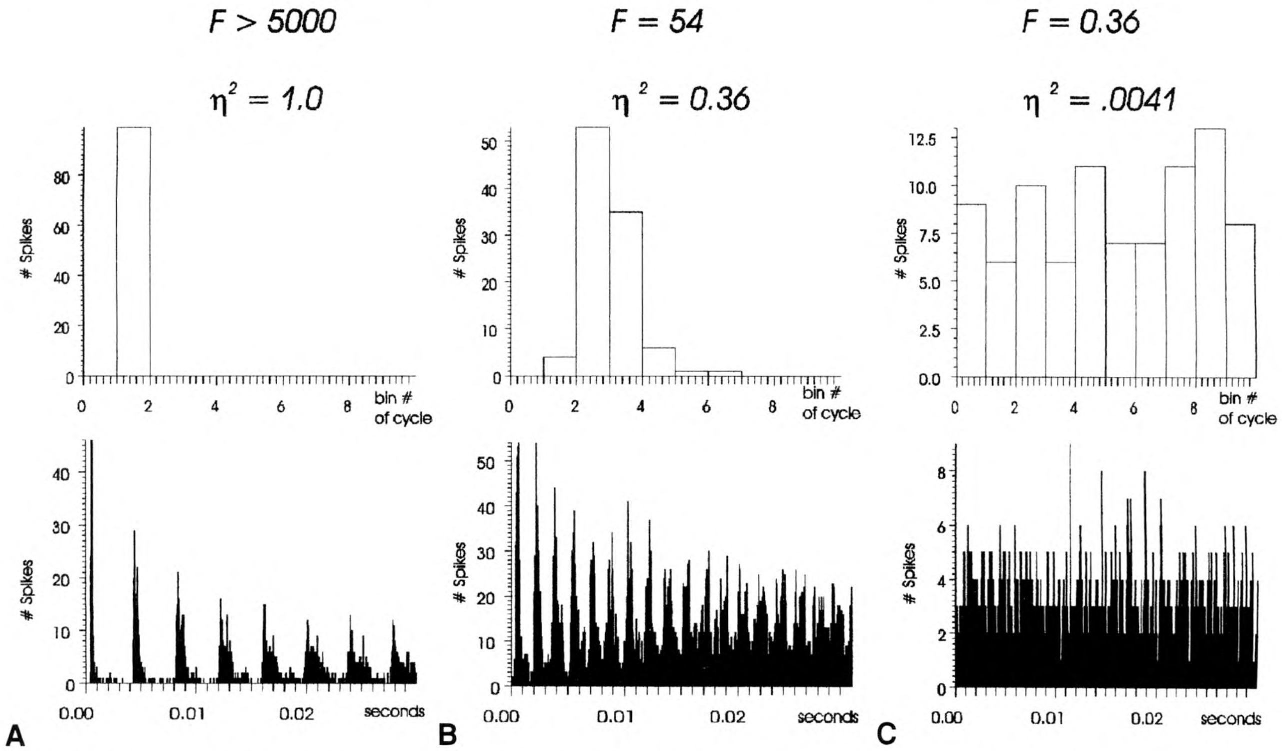

Fig 9. Frequency-following cross-correlation analysis. Analysis of variance F ratio, η^2 value, phase histogram, and cross-correlation graph are displayed for 2 examples of group 2, vibration-following single-unit fibers as compared with 1 example of group 3 fiber, which does not follow vocal fold vibratory pattern. **A**) Fiber with very tight following reflected by η^2 value of 1.0. Upper graph is phase histogram, and lower is cross-correlation graph. **B**) Example that does not seem to follow as well. Difference in number of peaks per unit of time in lower cross-correlation graph is due to difference in fundamental frequency of phonation between 2 animals. (Fiber in A has fundamental frequency of 180 Hz, while fiber in B is above 650 Hz.) **C**) Group 3 fiber that has maximum spike rate during phonation but that does not appear to follow vocal fold vibration pattern. This fiber is depicted in Fig 10.

the total number of afferent single units, it seemed reasonable to assume that a larger sampling could reveal even more groups. Evidence for this assumption is present in the form of 2 additional single units that were from 1 cat but showed very different activity patterns. The first single unit had a low, constant spike rate independent of respiration, phonation, or manipulation. The second single unit had spike rate patterns much different from those of any other isolated single unit (Fig 11). This single unit had peak spike rates at mid-inspiration during both control respiration and PAG stimulation. It did not respond to probing and failed to be quieted after prolonged administration of topical anesthetic. This single unit might serve receptors located deep within the muscle, tendon, or joint.

Silent Versus Tonic Activity. Sixteen silent and 44 tonic response types were identified according to published rate criteria.[1,2] There were 7 silent units in group 1 and 5 silent units in group 2, and all 4 of the group 5 single units showed rates of less than 10 Hz before PAG stimulation. The highest response rates of "silent" single units were seen during phonation in group 2, frequency-following single units (range, 140 to 300 Hz). Silent single units in groups 1 and 5 responded at rates of less than 175 Hz during PAG stimulation.

TRACHEAL PRESSURE DURING PHONATION

The pressure tracings obtained during phonation were seen to crescendo and decrescendo smoothly. No single units were identified that had an ImeanFr plot that was shaped similarly to the changes that occurred in tracheal pressure during phonation.

DISCUSSION

While there is considerable information in the literature concerning the behavior of laryngeal mechanoreceptors in response to a variety of mechanical and chemical stimuli, these activities have been studied largely with respect to reflexes that protect the lower airway or reflexes involved in airway patency and breathing. Nevertheless, these receptors are exquisitely sensitive to brief puffs of air, light touch, movement, stretch, and vibration. Since these are the same types of stimuli that occur during vocalization, it has been suggested that iSLN input might have a role in modulation of vocalization.[4-6] The present study supports this line of reasoning with results that

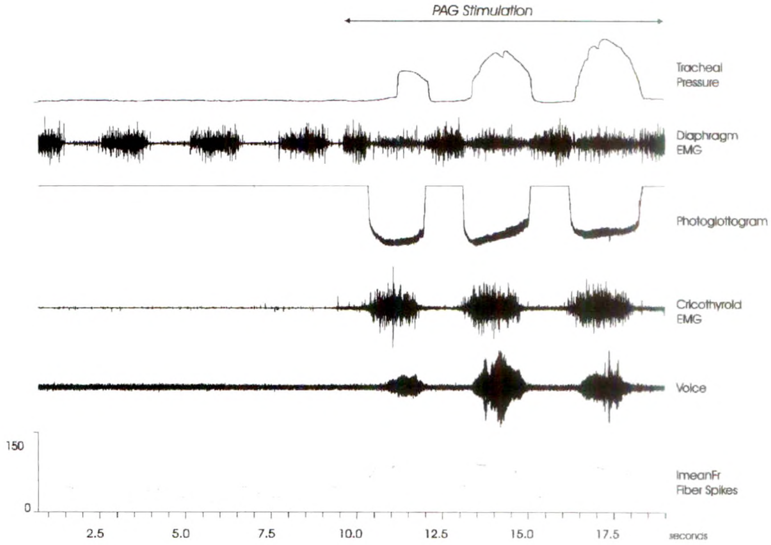

Fig 10. Group 3, expiratory, non–frequency-following single-unit fiber. Data channels reveal that these fibers had characteristic patterns of spike activity during control respiration. During PAG stimulation, spike rate increases from low levels that occur at time of vocal fold closure to peak that occurs just as phonation begins. Offset occurs late in phonation, after slight gradual decline in rate that is independent of volume of phonation.

demonstrate several modes of phonation-related responses from laryngeal mechanoreceptors served by the iSLN.

In the present study, iSLN responses were classified on the basis of effects that occurred during respiration and evoked vocalization. Comparison of the classification developed in this study with those published in the literature is difficult, since most other studies have not examined afferent activity during vocalization. Some of the manipulations used by other investigators to classify receptor activity (eg, laryngofissure in a paralyzed animal) could not be applied in this model. Nevertheless, we were able to test a limited number of receptors using mechanical probing and topical anesthesia. Pressure manipulations, as done by Sant'Ambrogio et al,[5] were not done because of the difficulty of maintaining a seal at the nose and mouth with the stereotaxic head holder and fiberoptic scope in place. In addition, we reasoned that the negative pressure responses that have been seen in the majority of iSLN afferents are not important during phonation, in which positive pressure changes are important. Positive pressure responses are relatively less common, and the negative pressure receptors are only minimally affected by positive pressure.[3,5,20,24] Since the animals were paralyzed, the pressure manipulations caused passive movements of the structures. In fact, Mathew et al[20] reanalyzed a group of receptors that they initially thought were responding to pressure changes and found that 51% of the negative and 33% of the positive pressure receptors maintained a respiratory modulation even during tracheotomy breathing. That this "drive" component was attenuated or eliminated by paralysis confirms its sensitivity to movement.

Tracheal pressure, or stretch, receptors located in the posterior wall of the trachea[25] share some characteristics with laryngeal pressure receptors. Pres-

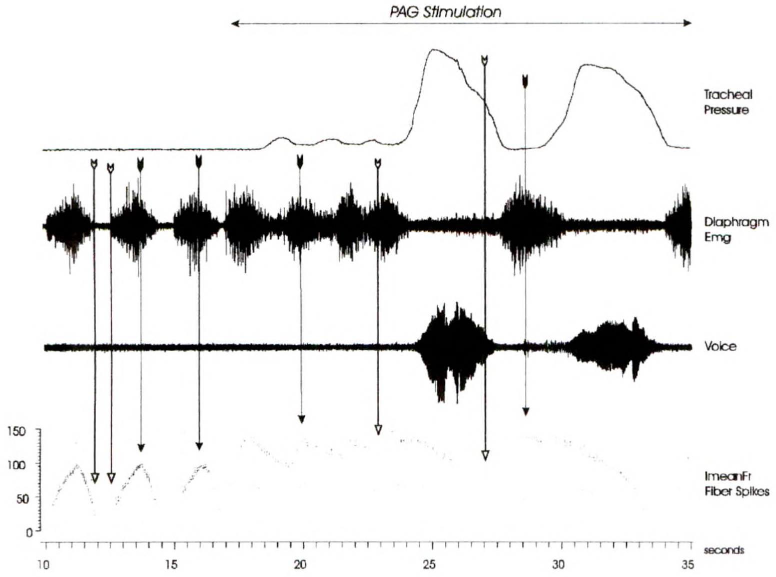

Fig 11. Miscellaneous single-unit fiber with spike rate patterns much different from those of any other fiber isolated. Peak spike rates occurred at mid-inspiration during both control respiration and PAG stimulation. Arrows are positioned to help reader compare data across channels. White arrows indicate examples of low spike activity. Black arrows point to peak spike rates.

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sure receptors in both locations are usually tonically active and slowly adapting. Most of the single units found in the present study were also tonically active, but it was not possible to quantify adaptation rates. Hwang et al stated that pressure changes "may activate receptors by mechanical distortions of the laryngo-pharyngeal walls."[26(p365)] Bartlett et al[27] suggested that differences in receptor responsiveness are due to some mechanical arrangement of the receptor microenvironment. Differences between the several classes of laryngeal mechanoreceptors might be explained by the concept that mechanical factors due to location are responsible, rather than some special feature of the receptors themselves.

We examined receptor activities by comparing the shape of the ImeanFr tracing of single-unit spikes with the shape of the pressure tracing. There were no cases in which the shapes were similar. The tracheal pressure peaked during the middle of phonation. Group 1 single units were not pressure-sensitive, since their activity peaked during the prephonatory phase. Their activity appeared to more closely parallel CT contraction, possibly reflecting vocal fold tension or stretch. Group 2 single units followed vocal fold vibration. Group 3 single units, such as the one depicted in Fig 10, showed a peak response well before the peak pressure occurred. Figure 10 shows an example of a series of 3 phonations, each with a peak pressure higher than the previous one. That the peak level and shape of the ImeanFr tracing is unchanged suggests a lack of response to tracheal pressure. Group 4, inspiratory-modulated single units could have been responding to negative pressure. The mixed group, group 5, responded with peaks in activity in several phases. These could be following both negative and positive pressures. The pressure responses of many laryngeal receptors reported in the literature may be due to mechanical displacement of structures, rather than pressure per se. During phonation, the mechanical aspects of vocal fold positioning, tension, and vibration may overshadow these mechanical factors.

Our data confirm the sensitivity of SLN single units to laryngeal movements, but we find no evidence of pressure-sensing activity during phonation. The absence of data to support pressure-responsive receptors is consistent with the findings of Garrett and Luschei,[10] Garrett,[28] and Mortola et al,[29] who reported that reflexes associated with expiratory airflow appear to act independently of positive pressure in cats.

Adaptation rates of touch receptors that responded to gentle stroking with a saline-soaked cotton wool pledget have been measured by clamping a blunt glass probe for 20 seconds to the receptive field and measuring the time required for the single unit to stop firing.[1] This approach to classification was used by Boushey et al[1] and Davis and Nail[2] and identified 2 groups. "Group 1" single units exhibited little or no spontaneous activity (<10 Hz) and variable adaptation rates. "Group 2" single units were tonically active (10 to 80 Hz, peak to 40 to 450 Hz during probing with a thread) and slowly adapting, and are thought to represent the touch receptors of Sampson and Eyzaguirre.[4] Quantification of adaptation rates requires mechanical stimulation over long periods of time, which was not possible with our setup; therefore, classification was not made on the basis of adaptation. The majority of single units in the present study were tonically active and could be classified as the tonic single units of Boushey et al and Davis and Nail. Boushey et al used paralyzed, ventilated cats, thus obviating the possibility of identifying respiratory modulation. Many of the laryngeal receptors in our study were very sensitive to muscle activity and movement, so it is possible that many receptors classified as silent single units might become tonic single units in a nonparalyzed animal. Davis and Nail further characterizated these 2 classes of single units using paralyzed, ventilated animals. They confirmed the sensitivity that both classes showed to touch and vibration and confirmed differences in adaptation rates. In their discussion, they suggest the possibility that forces exerted by the "open-larynx preparation" could have caused some of the tonic activity patterns observed. In support of this concept, they note how easily probing in some silent single units could produce tonic activity. Considering that 57 of 60 single units showed an increase in spike rate during some phase of evoked vocalization, there were clearly many potent stimuli present during our experiments that would be absent if the animals were paralyzed and ventilated. In analyzing our results, we found no clear-cut categories that might elevate our understanding of the system, except for those that related to changes in spike rate during phases of respiration and vocalization.

A significant incidence of inhibition of spontaneous single-unit discharge during vocalization was reported by Garrett.[28] With respect to vocalization, this finding is different from the present data. Eleven of the 35 units reported by Garrett exhibited a pause response during phonation. All of these units showed an increase in activity during inspiration. The majority were tonically active during respiration. We did not identify this type of activity. The majority of single units in the present study showed an increase in rate at some point during the PAG stimulation sequence. Two of the 3 inspiratory-modulated (group

4) single units showed no change in rate between inspirations during PAG stimulation (INSP-VOC) and control respiration phases (INSP-CONT). The third single unit had a 4.5-times-augmented rate during PAG stimulation inspiration (INSP-CONT) and responded during EXP-VOC and VOC, as well. It is possible that Garrett experienced temporary loss of contact between the nerve single unit and the recording electrode during phonation. We were very cautious to provide enough slack in the nerve to prevent this. Sampling differences could also explain this discrepancy.

Several types of responses during vocalization were characterized. Group 1 single units had peak activity during the prephonatory phase of vocalization. These single units could provide feedback during the prephonatory phase when the vocal folds were positioned, tightened, and readied for the production of a certain pitch. Here, auditory feedback was not operational, since no sound had been produced. The response of group 1 single units could be due to vocal process contact or stretching of epithelium, either along the vocal folds or in locations removed from the glottis. Our hypothesis was that feedback from this single-unit group might be used by the central nervous system (CNS) during the prephonatory phase, as suggested by Wyke's hypothesis.[8,30,31] On the other hand, group 1 single units might be monitoring some aspect of CT muscle activity. It was also possible that activity, which peaked during the prephonatory phase (or any other phase), could be unrelated to phonation. Group 2 single units (frequency-following) might then help maintain the chosen pitch. Group 3 single units showed activity peaks during phonation without following frequency. These single units might monitor changes occurring around the epiglottic joint to provide laryngeal position information as the larynx is elevated and depressed by the extralaryngeal muscles and the action of the CT muscles. Group 4 single units were inspiratory-modulated during vocalization and might help monitor vocal fold abduction that occurs during rapid inspirations taken between vocalized phrases. Group 5 single units were both inspiratory- and expiratory-modulated during vocalization. The patterns of inspiratory versus expiratory modulation obtained during evoked vocalization often differed from those obtained during control periods. It was not unusual to see a single unit, for example, that could be defined as inspiratory-modulated during quiet respiration (Fig 4) become intensely expiratory-modulated during evoked vocalizations. This finding further confounds the problem of classification.

The results of one study involving manually applied pressure stimulation to various laryngeal sites suggest that most of the receptors studied are located outside the muscles, in ligaments and around joints.[32] Several investigators have stated that most of the laryngeal receptors are not positioned deep within the laryngeal muscles, tendons, or joints, but rather, seem to be located superficially in the laryngeal mucous membrane, since the majority become unresponsive after light topical anesthesia.[1,4,6] Two of the 3 receptors tested in the present study were silenced by anesthesia. The reasons for this low number of single units tested with topical anesthetic included several experiments with results that could not be analyzed. In addition, since the experiment had to be terminated after placement of the anesthetic, it was always difficult to know when the last viable single unit was being examined. Because of the low yield of single units per nerve, the most common situation was that there were no more viable single units before the decision was made to terminate the experiment by applying topical anesthetic.

Although the results of the laryngeal anesthesia experiments in humans and in animals do support the hypothesis that laryngeal mechanoreceptor activities play a role in control of phonation under some circumstances, the currently available data may either understate or overstate such contributions. First, there is evidence suggesting a role for extralaryngeal vagal afferent proprioceptive control of laryngeal activity.[9] Second, signals from proprioceptors may be carried by the recurrent laryngeal nerve[33] and the external branch of the SLN[34] and therefore be left intact in experiments involving SLN blockade. If several distinct channels operating in parallel signal proprioception, then elimination of only some of them, under reported conditions of anesthesia, may not be sufficient to completely negate this proprioception. Third, muscle spindles or other deep receptors could have been affected by the prolonged topical anesthesia. Fourth, these studies do not provide data on the adequacy or persistence of anesthesia, although some describe elimination of protective reflexes upon laryngeal probing as an end point. Fifth, auditory feedback was operational during these studies and might have obscured the effects of the anesthesia. Finally, it is possible that the topical anesthesia may have had direct effects on the laryngeal muscles themselves.

Other investigators have probed the larynx in an attempt to localize receptors.[1,2,4,6] This method is more useful when the larynx is split open, as it is after laryngofissure, and the animal is paralyzed. The responses to probing were difficult to interpret in the present study. Probing also excited laryngeal protective reflexes that often resulted in laryngeal movements such as cough. Displacement of the epiglottis with the tip of the fiberoptic scope was the easiest probing maneuver. Several single units from each group identified responded to epiglottic displacement. These receptors might be located on the epiglottis or in the epiglottic joint. It is also possible that displacement of the epiglottis stretches the tissues and stimulates remotely located receptors. Although many receptors are anatomically located such that they might respond to epiglottic displacement,[2] the response was not a universal finding in this study. In group 1, 2 of 4 single units responded to epiglottic displacement, while in group 2, 2 of 5 single units responded. All 3 of the receptors in group 3 responded to epiglottic displacement and showed a characteristic pattern of response during control and PAG stimulation. These receptors may be located within the epiglottic joint; however, accurate anatomic localization of the receptors within each group requires further work.

Our data indicate that a proportion of the afferent traffic to the CNS that occurs during vocalization concerns the vocal fold vibratory pattern. This demonstration of vocal fold vibration frequency–following during vocalization corroborates the findings of Garrett,[28] who described a similar correlation in 4 single units between glottal pressure pulse frequency and afferent spike rate, and with those of Miura et al,[35] who reported that 13 of 14 single units responded during vocalization with a discharge frequency proportional to the fundamental frequency of the voice. This is a very high incidence as compared to our experience and to that of Garrett. The response pattern of these receptors suggests that they represent a specific vocal fold vibration–sensing group. One would predict that most classes of laryngeal mechanoreceptors would respond during phonation to vocal fold vibration, and we identified both "silent" and "tonic" single units similar to those of Boushey et al[1] and Davis and Nail,[2] which we classified as frequency-following. Although Davis and Nail failed to find any receptive fields on the membranous vocal folds, in fact, many receptors exist in the densely innervated vocal process area.[2,36] These receptors might be expected to respond during vocal fold contact in the prephonatory period or during attempted phonations that produce complete vocal fold closure *without* voicing, as was seen with the group 1 single units. One might also expect these receptors to respond to vocal fold vibration. However, although the group 1 and group 3 single units were expiratory-modulated during vocalization, they did not follow the vibratory pattern of the vocal folds. This suggests some specificity in the response of the group 2, frequency-following single units. The majority of single units within the larynx are capable of responding to mechanical oscillations.[2,4] Sampson and Eyzaguirre[4] stated that the frequency and amplitude of the vibration had to be *critically adjusted* to yield 1:1 following. We suggest that the conditions that must exist for group 2 single units to be activated are optimized by muscle positioning and tensing and by the vibratory activity of the vocal folds during phonation. This specificity may be due to the particular microanatomic environment of these receptors. Inspection of the PGG and CT EMG tracings made during PAG stimulation revealed no group 2 single-unit activity during the prephonatory phase of expiration. Thus, group 2 single units are not simply responding to laryngeal muscle contraction during adduction or vocal fold contact or when the folds are held tightly together during the prephonatory phase of expiration, as might be expected for touch receptors. It appeared that vocal fold vibration during phonation was the required and necessary stimulus for activation of group 2 single units.

Sampson and Eyzaguirre[4] also noted that better following seemed to occur with a higher-amplitude vibration stimulus. Similarly, in the example presented in Fig 6, frequency-following activity during this loud vocalization shows a high proportion of 1:1 following. Subsequent quieter, lower-pitched vocalizations showed no 1:1 following, with most spikes following at 1:2 or less. There were also many instances in which the PGG showed a complex, intermittently irregular vocal fold vibratory pattern. In these instances, the single unit appeared to be accurately following these subtle vibratory perturbations (Figs 6 and 8). The η^2 value and ANOVA statistical analysis provide quantification of the degree to which each frequency-following single unit's spike occurrences are phase-locked to the vibratory cycle. It appears from the current data that group 2 single units are potentially able to code for vocal fold vibration rate (pitch).

Our results indicate that some laryngeal afferents respond to phonation-specific events during vocalization. This stereotyped afferent information might be used by the CNS to modulate vocalization. Further evaluation of the effect of these responses on CNS function will be necessary for determining the importance of this information for the regulation of voice.

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II. EFFECT OF RECURRENT LARYNGEAL NERVE PARALYSIS ON SUPERIOR LARYNGEAL NERVE AFFERENTS DURING EVOKED VOCALIZATION

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We tested the hypothesis that vocal fold paresis leads to a substantial reduction in activity from the internal branch of the superior laryngeal nerve (iSLN) during respiration and evoked vocalization. The iSLN afferent activity was measured before and after recurrent laryngeal nerve paresis by cold block in decerebrate cats during spontaneous respiration and electrically evoked vocalization. Response rate patterns of 33 iSLN single units from 11 cats were categorized into 5 groups based on responses to vocalization. Only 24% of single units during spontaneous respiration and 18% during evoked phonation displayed activity pattern changes as a result of paresis. Those fibers affected were heterogeneous in discharge pattern, but none of the units that followed voice frequency lost this characteristic when the motor nerve was cooled. The relative insensitivity of iSLN activity to motor paralysis suggests that the receptors studied are coupled to tissue such that passive interaction rather than active muscular contraction is the major stimulus.

KEY WORDS — cat, decerebration, laryngeal receptors, respiration.

INTRODUCTION

Vocal fold paresis is a common laryngeal disorder most often caused by a neurotropic viral infection or as a result of recurrent laryngeal nerve (RLN) injury during neck or chest operations. Patients with a paralyzed vocal fold experience chronic aspiration during swallowing. They also have a characteristic voice quality that can be partially alleviated by a vocal fold medialization procedure.[1] It is unknown how unilateral RLN paresis changes sensory input to the central nervous system during respiration and phonation.

The larynx is richly supplied with mechanoreceptors in the superficial vocal fold and supraglottic epithelium.[2-5] These receptors participate in protection of the lower airway through the glottic closure reflex and coughing. They also play a role in regulation of airway patency and breathing.[4,6,7] Laryngeal mechanoreceptors respond to the types of stimuli that occur during phonation,[2,3,8] and phonation-specific afferent activities can be recorded in the internal branch of the superior laryngeal nerve (iSLN) during evoked vocalization in cats.[9,10] Mechanoreceptor stimuli occurring during vocalization include mucosal contact, vibration, and laryngeal movements.[2-4] Most laryngeal mechanoreceptors also respond to a maintained negative or positive pressure in the isolated upper airway, and thus, pressure is considered to be a major stimulus for laryngeal mechanoreceptors.[4,6,11-15] However, laryngeal movements also produce important stimuli for these receptors, since many putative pressure-sensing receptors are in fact responding to vocal fold movement.[14]

To the extent that muscle-driven vocal fold movements create critical stimuli for iSLN receptors, it is difficult to predict their physiological behavior from experiments in which systemic paralysis or laryngofissure has been used.[2,3,16,17] In addition, the types of manipulation used to characterize mechanoreceptor responses[2-4,18] cannot duplicate the complex laryngeal behaviors associated with natural vocalization. Investigators have noted loss of laryngeal iSLN receptor responsiveness after RLN section in otherwise nonparalyzed, nonvocalizing animals.[4,14,15] Whether this pattern of responses would carry over to conditions of vocalization is uncertain. Laryngeal behaviors seen during vocalization are much more vigorous than those seen during quiet respiration. Airflow, pressure changes, and movements of the mobile laryngeal structures during vocalization might stimulate receptors in the paralyzed vocal fold. The anatomic configuration of laryngeal structures is such that receptors can be affected by distant stimuli,[3] and many vocal fold mechanoreceptors are located posteriorly, near the arytenoid cartilages, so that forces due to movements by the intact side can easily be transmitted to them.[18] When the ipsilateral external

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branch of the superior laryngeal nerve (SLN) remains intact, action of the cricothyroid (CT) muscle also contributes to the tension and adduction of the paralyzed vocal fold.

In order to investigate the effects of vocal fold movement alteration on response patterns of iSLN afferents, we measured iSLN afferent activity before and after RLN paralysis by cold block in decerebrate cats during electrically evoked vocalization. We tested the hypothesis that vocal fold paresis leads to a substantial reduction in iSLN afferent activity during respiration and evoked vocalization.

METHODS

General. All procedures were performed under the guidelines and approval of the University of Oklahoma Institutional Animal Care and Use Committee in compliance with "The Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 85-23, revised 1985). Repetitive vocalizations were produced in decerebrate cats by stereotactically guided electrical stimulation of the midbrain periaqueductal gray (PAG). Single-unit recordings were made from isolated nerve bundles of the distal stump of the sectioned iSLN during vocalizations. Simultaneous recordings were made of the voice, the photoglottograms (PGGs), and the diaphragm and CT electromyograms (EMGs) before and after cold block of the ipsilateral RLN.

Anesthesia. The cats were first tranquilized with intramuscular ketamine hydrochloride (10 mg/kg). Then, 1% Xylocaine with 1:100,000 epinephrine was injected into the groin, and femoral vein and artery catheters were placed for drug administration and blood pressure monitoring, respectively. A deep plane of anesthesia was established with halothane via an endotracheal ventilating tube. Diaphragm electrodes were then placed, and the animal was positioned in a stereotaxic head holder. After craniotomy, a supracollicular decerebration was done by suction aspiration. The halothane administration was discontinued, and the endotracheal tube was removed when the vital signs became stable. Valium (0.3 to 1 mg/kg) was used to decrease the animal's irritability. All animals received 2 mg of dexamethasone acetate. The blood pressure was measured with a pressure transducer connected to the femoral artery catheter. The rectal temperature was controlled (37°C ± 1°C) by an automatic heating blanket. At the end of the experiment, the animals were euthanized by a pentobarbital sodium overdose (>200 mg/kg).

Vocalization. A stimulating electrode was first positioned at stereotaxic coordinates of zero in the anterior-posterior plane and 1.5 mm lateral in the medial-lateral plane. Then, the electrode was positioned in the dorsal-ventral plane at 0.5-mm increments to the range of 15 mm above the interaural plane until the strongest phonation was produced with the least stimulating current. The expected electrode placement based on the stereotaxic coordinates was in the general region of the caudal midbrain PAG. Stimulating parameters of 0.1 to 1 mA, 200-μs pulse duration, at 100 Hz were used to produce vocalizations.

Nerve Dissection. A neck incision was made, and the skin was used to contain a pool of warm mineral oil (39°C). The iSLN was transected proximal, the neural sheath was removed from the cut end, and bundles of the nerve were teased apart. Distal nerve bundles were placed over platinum bipolar hook electrodes immersed in mineral oil. Fixation of the electrode complex to the laryngeal skeleton with cyanoacrylate cement and epoxy allowed the electrodes to move with the nerve. This design helped prevent the nerve from being pulled off the electrode complex when the animal swallowed, coughed, or vocalized.

Peripheral Nerve Recording. Action potentials from iSLN nerve slips were high-pass fourth-order filtered at 20 Hz, amplified with a preamplifier (model DAM-5A, World Precision Instruments, Sarasota, Fla), displayed on an oscilloscope and an audio monitor, and digitally recorded.

RLN Cold Block. The right RLN was identified in the tracheoesophageal groove, exposed, isolated, and covered by warm mineral oil contained by skin flaps. A U-shaped copper pipe was passed around the nerve, and ice water was pumped through it in order to cool the nerve to between 2°C and 5°C. Paresis was considered complete when, while directly observing the larynx though a flexible fiberoptic endoscope, we could see that spontaneous and evoked vocal fold movements had ceased.

Monitoring Vocal Fold Movements. The tip of a flexible fiberoptic endoscope (Olympus EnFP-2, Hitschfel Instruments Inc, St Louis, Mo) with an attached video camera was passed transorally and suspended inside the hypopharynx, above the laryngeal inlet. The endoscope illuminated the larynx from above and served as a light source for the PGG. Photoglottography is a technique that uses a photoelectric transducer to describe time-varying changes in glottal area.[19] Light from the endoscope aimed through the glottis was transduced by a photosensor (OSD-60, Centronics Inc, Newbury Park, Calif) with

TABLE 1. CATEGORIES OF iSLN AFFERENT ACTIVITY SEEN DURING EVOKED VOCALIZATION

	Modulation	*Activity*
Group 1	Expiratory-modulated	Show peak activity before phonation or during early phonation, without following vocal fold vibratory frequency
Group 2	Expiratory-modulated	Follow vibratory frequency of vocal folds
Group 3	Expiratory-modulated	Active during phonation without frequency-following, and not activated before phonation
Group 4	Inspiratory-modulated	Active during inspiration only, and quiet during phonation
Group 5	Miscellaneous	Show variety of activity patterns, including tonic, bursting, and vocal fold movement–following patterns

iSLN — internal branch of superior laryngeal nerve.

a custom-built amplifier, placed on the neck just below the cricoid cartilage. The PGG signals were monitored on an oscilloscope to assess the adequacy of the glottographic signal. The PGG data were used to indicate vocal fold movements.

Recording Phonation. Acoustic data were acquired via a custom-built amplifier and microphone (model UA0196, Bruel and Kjaer, Copenhagen, Denmark) placed 15 cm from the mouth. This gave an indication of the onset and offset of phonation and the relative intensity. The output from the microphone was digitally recorded and monitored with an oscilloscope.

Electromyography. The EMG activity of the diaphragm and CT muscles was monitored with bipolar electrodes placed into the respective muscles. The electrodes were prepared by removing 2 to 3 mm of insulation from a 30-gauge stainless steel wire and passing the bared end of the wire just through a 25-gauge hypodermic needle. The bared portion of the wire was bent approximately 140° to create a hook that was then passed into the target muscle and remained anchored after the needle was removed. The costal diaphragm muscle was exposed through a midline skin incision over the xiphoid process. The 2 hook-wire needle electrodes were passed just behind the xiphoid process, parallel to the plane of the diaphragm muscles, under direct vision. This placement minimized contamination of the diaphragm EMG by intercostal muscle activity. The electrodes were placed into the CT muscle under direct vision. Each EMG was amplified (model 7DAG, Grass, West Warwick, RI), viewed on the oscilloscope, and recorded digitally.

Classification of iSLN Afferent Activity. The activity of iSLN single units was examined during spontaneous respiration and evoked vocalization sequences. All single units were classified primarily as to how the action potential rate (spike rate) changed during PAG stimulation (evoked vocalization sequence) and classified secondarily during spontaneous respiration. Spontaneous respiration and evoked vocalization sequences were divided into inspiratory and expiratory phases. Inspiration was defined by eye as starting at the onset of diaphragm EMG and slight vocal fold abduction (seen on the PGG) and ending as diaphragm EMG activity began to fall from its maximum level. Expiration was defined as starting with the decline of the diaphragm EMG, as well as vocal fold adduction (seen on the PGG), and ending as the next cycle of diaphragm EMG activation began. The expiratory phase of vocalization was further divided into prephonatory and phonatory phases.

Visual inspection of the raw record and the instantaneous mean frequency (ImeanFr) tracing over time was used to determine a significant spike rate change during each phase. The ImeanFr is the reciprocal of the time between 2 spikes. The ImeanFr tracing displayed spike-to-spike changes in firing rate. Single-unit responses were categorized into 5 possible patterns of activity (Table 1). The spike rate of group 1 single units showed a peak response during the prephonatory expiration phase or at phonation onset; then, the rate dropped to a plateau and generally remained constant until the next inspiration. The maximal activity occurred during PAG stimulation, when the cat closed the glottis but did not produce enough effort to phonate. Group 2 single units had onset of activity and maximum activity during phonation. They were recognized by high spike rates (>150 Hz) and by harmonics displayed on the ImeanFr plot. These single units followed the vibration frequency of the vocal folds. Visual inspection of the timing of voice waveform peaks and single-unit spikes was used to identify a consistent relationship in order to prove frequency-following activity. Group 3 single units showed peak activity during phonation that did not follow vocal fold vibration or peak during the prephonatory phase or early phonation. Group 4 single units were active only during inspiration during both control and PAG stimulation conditions. In this study, group 5 included those single units with all other patterns of activity. The majority of group 5 single units showed tonic rates (<50 Hz) with slight phasic rate changes associated with vocal fold movements during respiration and slight rate increases during phonation.

Criteria Used to Screen Recordings. The iSLN

TABLE 2. EFFECTS OF COOLING RLN ON iSLN AFFERENT ACTIVITY BY GROUP CATEGORY

Group Category	*No. of Single Units (n = 33)*	*Change on Cooling*		
		Respiration	*Phonation*	*Both*
Group 1 (peak before phonation)	7	2	1	1
Group 2 (frequency-following)	5	0	0	0
Group 3 (expiratory)	9	1	1	1
Group 4 (inspiratory)	5	0	0	0
Group 5 (miscellaneous)	7	1	0	2

RLN — recurrent laryngeal nerve.

nerve slips were divided into small bundles in order to obtain a recording of action potentials from a single axon (single unit) or several axons (multiunit). When a single unit recording resulted, the amplitude of the spike was well above the noise level and the ImeanFr tracing created a waveform-like pattern. When spikes from several axons were seen, the computer selected them by spike shape and amplitude using identification software (Spike 2, version 4.0, Cambridge Electronic Design, Cambridge, England). The acceptability of the result was judged by visual inspection of the raw data channels and the ImeanFr tracing. The ImeanFr tracing was more useful than a histogram of average rate with 0.1-second bins, because most of the subtle spike-to-spike changes were lost in the histogram but were visible on the ImeanFr plot. The ImeanFr plot allowed recognition of complex patterns of activity, which were lost in the histogram type of display. In addition, there were instances in which the activity of a specific group of single units could be visualized on the ImeanFr plot in spite of the surrounding scatter of points due to multiunit spikes.

Recordings were considered adequate when the respiratory sequence contained at least 3 inspirations and the evoked vocalization sequence contained 1 to 3 phonations. When possible, 2 sequences before and after cooling of the RLN were recorded. When a spike disappeared after cooling of the RLN, it must have returned on the next recovered (before cooling) sequence in order to be considered valid.

Recordings were rejected when 1 of the following conditions occurred: 1) phonations were not easily visible on the audio channel, were inconsistent, or were not induced; 2) all single-unit spikes disappeared during the stimulation sequence; 3) the spike amplitude had a greater than 20% change before and after cooling; 4) the templates or inspection could not separate 2 spikes of similar amplitude; 5) the spike rate was irregular and the amplitude was low, such that it became difficult to separate spikes from baseline noise and/or an active larger-amplitude spike; 6) the stimulus artifact obscured visual inspection of the raw record and a poor ImeanFr waveform was present; 7) a poor ImeanFr waveform occurred in which there was not a smooth change in rate from spike to spike (this was seen as a scatter of points rather than a pattern that approximated a curvilinear-shaped line or waveform); or 8) inconsistent ImeanFr patterns occurred across phonations within a recording.

Criteria Used to Determine RLN Cooling Effect. Changes due to cooling of the RLN were considered significant when the peak, plateau, or trough spike rate within a respiratory or phonatory phase changed by 20% or greater after cooling. Because the phonation intensity and quality changed dramatically as a result of the vocal fold paresis, single units in the 2 expiratory-modulated groups (groups 1 and 2) had to lose characteristic features in order to be considered altered. Group 1 single units were required to lose the crescendo-decrescendo pattern during early

TABLE 3. ACTIVITY PATTERNS OF EACH SINGLE UNIT TESTED AS SEEN DURING SPONTANEOUS RESPIRATION

Group 1 (n = 7)	*Group 2 (n = 5)*	*Group 3 (n = 9)*	*Group 4 (n = 5)*	*Group 5 (n = 7)*
Expiratory*†	Tonic	Inspiratory	Tonic	Tonic*†
Phasic follows PGG*	Tonic	Inspiratory	Expiratory	Expiratory (tonic)
Tonic†	Expiratory	Tonic	Tonic	Expiratory*† (tonic)
Expiratory*	Quiet	Expiratory	Tonic	Phasic follows PGG (tonic)
Quiet		Inspiratory*†	Inspiratory	Quiet
Expiratory		Expiratory*		Expiratory*
Phasic follows PGG		Tonic		Phasic follows PGG (tonic)
		Inspiratory†		
		Tonic		

PGG — photoglottogram.

*RLN cooling had effect on spontaneous respiration.

†RLN cooling had effect on evoked phonation.

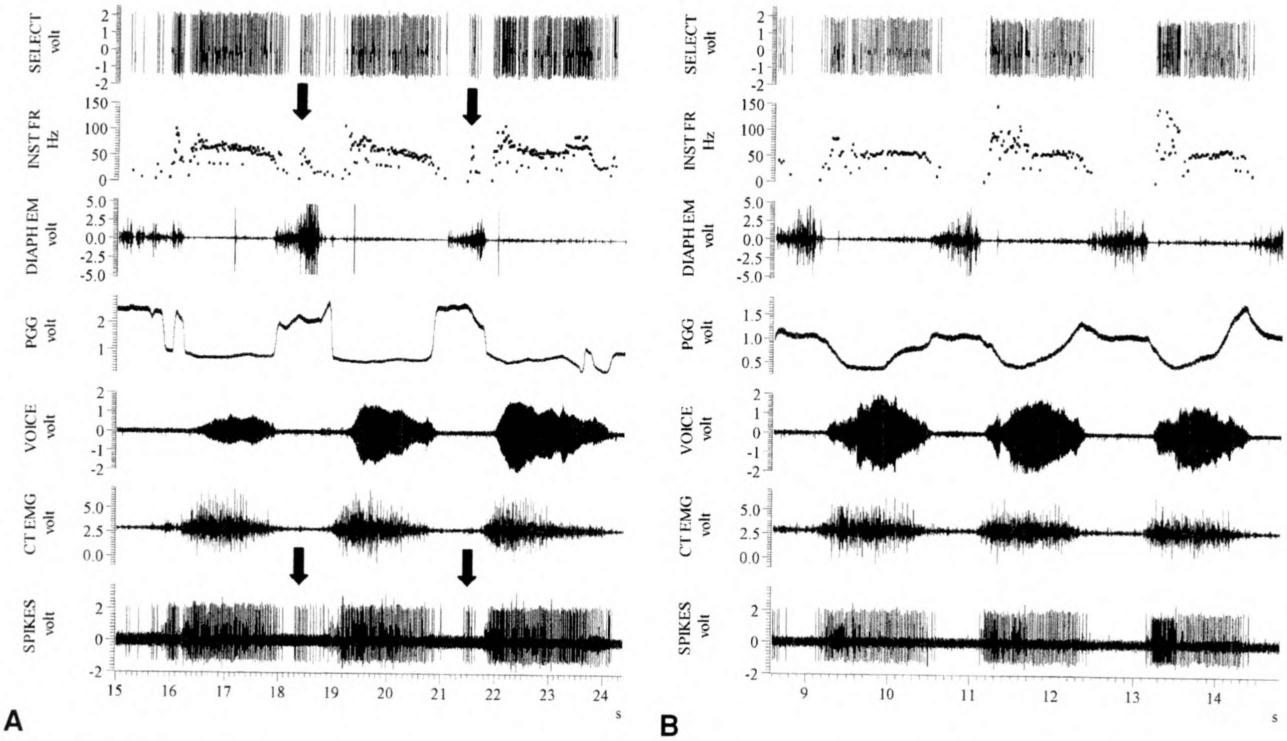

Fig 1. Group 1 single unit with lost inspiratory spikes after recurrent laryngeal nerve (RLN) cooling. Prephonation-peak activity pattern of this group 1 single unit was maintained during phonations, even after RLN cooling. **A)** Before cooling. **B)** After cooling. Late inspiratory burst present before RLN cooling (arrows) disappeared after cooling. Spontaneous respiration sequence for this single unit is shown in Fig 2. Photoglottogram (PGG) channel tracks abduction and adduction of vocal folds. SPIKES — raw action potentials of internal branch of superior laryngeal nerve (iSLN); CT EMG — cricothyroid electromyogram; DIAPH EM — diaphragm electromyogram channel; INST FR — instantaneous frequency of spikes selected by Spike 2 software template algorithm; SELECT — spikes selected by software.

expiration, and group 2 single units were required to lose frequency-following activity.

Data Analysis. The data were analyzed off-line by computer. Single-unit action potentials (spikes) and voice acoustic analog signals were sampled at 22 kHz, while the EMG and PGG signals were sampled at 11 kHz. The digitizer (Neurocorder, Neurodata Inc, New York, NY) saved the information onto a videocassette recorder that later played the signals to a computer-based waveform analysis system (Cambridge Electronic Design 1401-plus). All spike recordings were initially inspected visually. The spikes were sorted by a computer software spike recognition algorithm (Spike 2, version 4.0, Cambridge Electronic Design) that used amplitude and shape to differentiate the activity of a single unit from a population of single units. The software selected spikes that crossed a waveform signal trigger level to create templates based on spike shape. The template matching allowed an amplitude variation of 20% and required 60% of the data points to lie within the template for a single spike. These spike occurrences were further converted to electronic events with an associated ImeanFr tracing plotted against time. This plot was also inspected to confirm the presence of smooth transitions of frequency associated with single-unit activity.

RESULTS

Categorization of Single-Unit Response Patterns. Prior studies of iSLN afferents have often been done on paralyzed animals, and the categorizations have been based on respiratory activity or on manipulations that would be impossible in an intact preparation. The respiratory phase category was not predictive of the type of response seen during evoked vocalization. It was not unusual for a single unit that was inspiratory-modulated during spontaneous respiration to become expiratory-modulated during evoked vocalization. For this reason, and because the most vigorous response rates were seen during evoked vocalization, the primary categorization was based on responses seen during the evoked vocalization condition. The response rate patterns of 33 iSLN single units from 11 cats were categorized into activity groups 1 through 5. Graphic examples of group activity are presented in the following section.

Effects of RLN Cooling. The distribution of single units into groups and the number of single units affected by cooling of the RLN are listed in Table 2. Activity patterns seen during spontaneous respira-

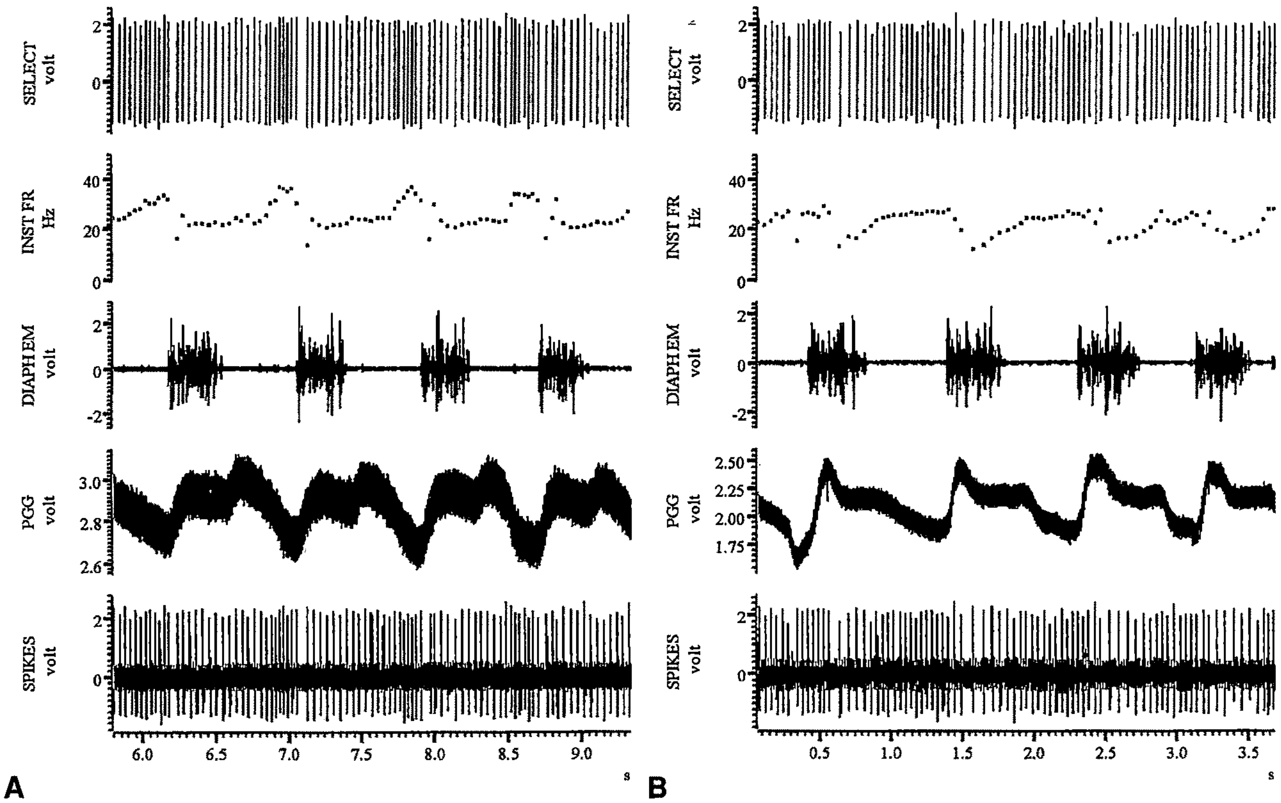

Fig 2. Group 1 single unit showing change during spontaneous respiration after RLN cooling. **A**) This group 1 single unit displayed rise in activity immediately before inspiration, during vocal fold abduction, across spontaneous respiration sequence. **B**) After cooling, this activity was lost. Evoked vocalization sequence for this single unit is shown in Fig 1.

tion did not predict which group the single unit fell into during evoked vocalization. There was no consistent activity pattern during spontaneous respiration among those affected by RLN cooling. Activity patterns for all single units tested during spontaneous respiration and the effects of RLN cooling are presented in Table 3. The vertical columns in Table 3 show the responses of all single units within a group. Those that changed activity with RLN cooling are indicated. The major activity pattern during spontaneous respiration is noted for each single unit tested.

Recurrent laryngeal nerve cooling effects were seen in 2 cases during phonation and in 3 cases during spontaneous respiration within the expiratory-modulated groups 1 and 3. Two cases were found in the expiratory-modulated groups that showed RLN cooling effects during both spontaneous respiration and evoked vocalization. There were no examples of RLN cooling effects seen in the expiratory-modulated, frequency-following group 2, nor in the inspiratory-modulated group 4. One group 5, miscellaneous single unit showed a change during respiration only, and 2 were changed during both spontaneous respiration and evoked vocalization after RLN cooling.

Group 1, prephonation-peak single units (n = 7) responded with a crescendo-decrescendo rate increase at the time of either vocal fold closure prior to the onset of phonation or during early phonation (Fig 1). In this example, there was no RLN cooling effect on the overall activity pattern during phonation (see criteria in Methods). A late inspiratory burst seen from a single unit during evoked vocalization was eliminated by RLN cooling. During spontaneous respiration, the single unit showed a tonic activity pattern during expiration and a distinctive rise and fall in activity immediately before inspiration that appeared to coincide with vocal fold abduction. This rise-and-fall pattern became tonic with a drop-off of activity during inspiration after RLN cooling (Fig 2). A group 1 single unit with characteristic rate changes during the prephonatory phase of expiration as seen on the ImeanFr channel is shown in Fig 3.

This prominent activity pattern decreased to 3 spikes during the first phonation, was lost during the second phonation, and was less intense during the last 2 phonations after RLN cooling. There was also a 20% drop in the plateau rate during phonation as a result of RLN cooling. The activity of the same single unit was not affected by RLN cooling during spontaneous respiration. This single unit failed to qualify as group 2 because of the low frequency rate seen during phonation (100 Hz) as compared to the fundamental frequency of the voice (500 Hz) and the lack

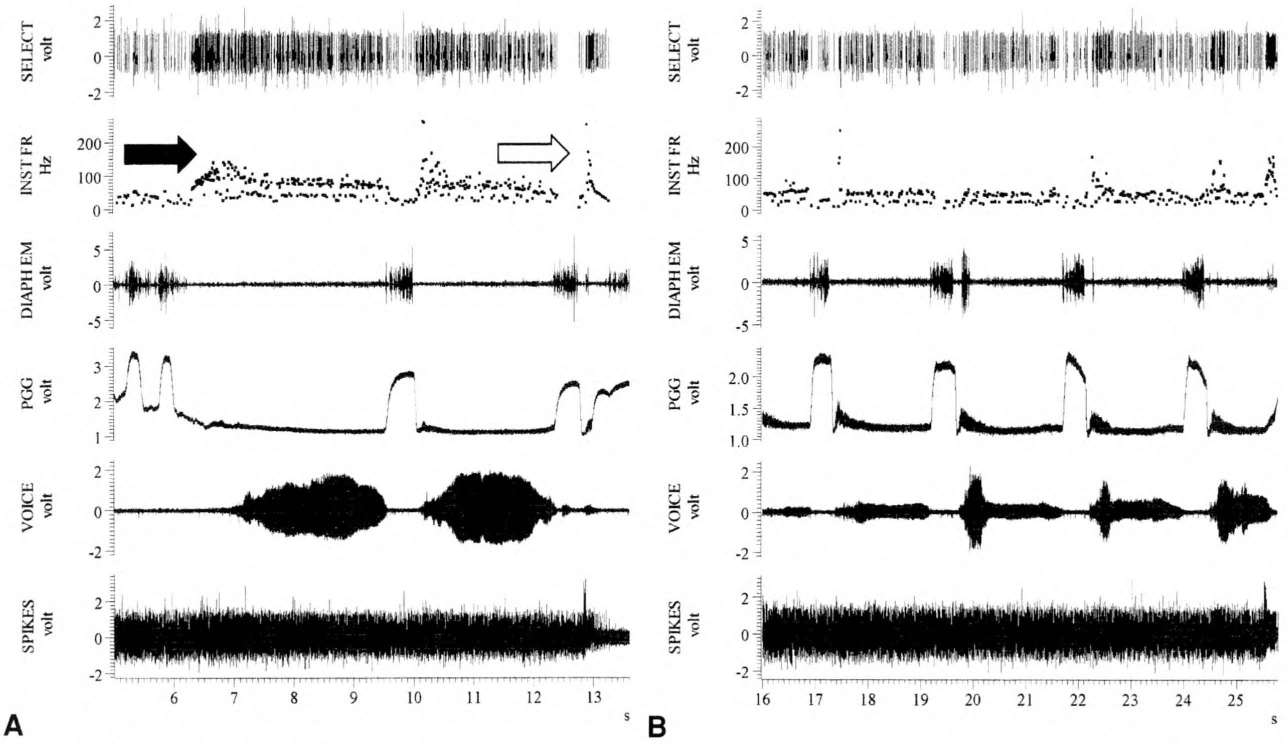

Fig 3. Group 1, prephonation-peak, expiratory-modulated single unit with RLN cooling effect. Characteristic crescendo-decrescendo rate activity change of group 1 iSLN single unit is seen during prephonation phase of expiration, during electrically evoked vocalization. **A**) Before RLN cooling. Black arrow — instantaneous mean frequency (ImeanFr) channel; white arrow — peak that occurred at vocal fold closure when animal failed to phonate. This peak is characteristic of group 1 single units. **B**) After RLN cooling. RLN cooling causes decrease in prominence of crescendo-decrescendo response during phonations. In addition, plateau during phonation is reduced by >20% after RLN cooling. Some multiunit activity is present in this example.

of a tight correlation with voice waveform peaks. One group 1 single unit lost respiratory modulation after RLN cooling during spontaneous respiration only.

None of the group 2 single units showed changes in response during spontaneous respiration or evoked vocalization as a result of RLN cooling. One single unit classified as showing group 2, frequency-following behavior is displayed in Fig 4. This single unit showed tonic activity during spontaneous respiration and typical frequency-following behavior during phonation. This single unit fired, at times, at a rate equal to the fundamental frequency of the voice. At other times, it fired at a fraction of the rate of the fundamental frequency, producing several "harmonics." (White arrows point to 3 harmonics on the third phonation in Fig 4.) Although there are differences in the ImeanFr pattern seen before and after RLN cooling, the quality, intensity, and pitch profile of the phonations have also changed. Frequency-following information persists in spite of the effects of RLN cooling. Note that the spike occurrences are tightly time-locked to a specific peak on the voice waveform, as shown in Fig 5. Frequency following persists despite RLN cooling. An example of group 3 activity is shown in Fig 6. This group of single units has expiratory-modulated activity during evoked vocalization, with a plateau during phonation and a lack of frequency following. However, this group also contains single units with activity patterns that might fit into either group 1 or group 2 with less strict criteria. Some group 3 single units have a loose correlation with the voice waveform but have rates of less than 75 Hz and lack the harmonic pattern on the ImeanFr plot associated with frequency-following single units. Some group 3 single units also have weak peaks during the prephonatory phase or during early phonation, similar to those associated with group 1 single units. One group 3 single unit showed an RLN cooling effect with a 20% drop in rate during spontaneous respiration (Fig 6) and a dramatic loss of activity during evoked vocalization (Fig 7). A second group 3 single unit had mid-inspiratory to expiratory bursting during spontaneous respiration that was unaffected by RLN cooling. The evoked vocalization sequence shows the same ramp on activation during early phonation, but after RLN cooling, there is a new peak at end expiration that is increased 20% as compared to that seen before cooling. One group 3 single unit showed a loss of phasic responsiveness during spontaneous respiration after RLN cooling,

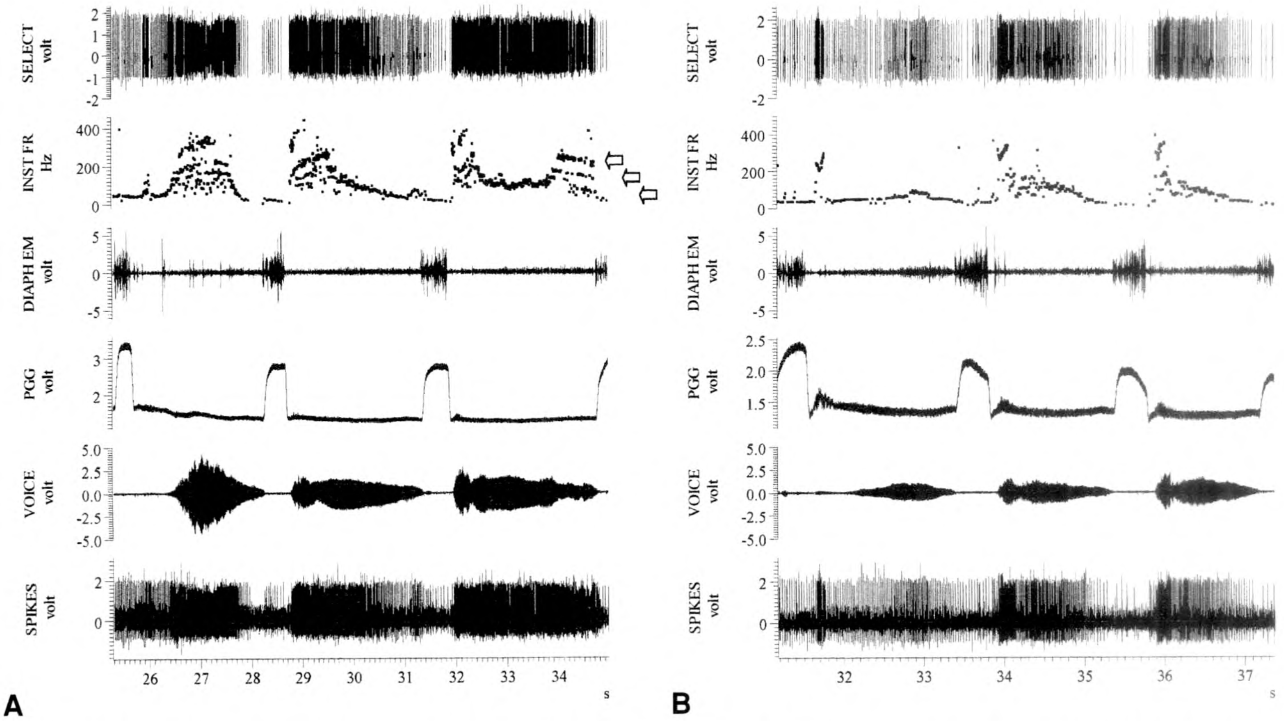

Fig 4. Group 2, frequency-following single unit without change after RLN cooling. Single unit shows typical higher-rate responses, with harmonics seen on ImeanFr plots during phonations. Activity during spontaneous respiration is tonic, without respiratory phasic variations. **A**) Before RLN cooling. Arrows — harmonics on third phonation. **B**) After RLN cooling. Although shape of ImeanFr plot is different, frequency-following characteristic is still present after RLN cooling. Changes seen were attributed to changes in phonation quality, intensity, and pitch caused by unilateral vocal fold paralysis. See Fig 5 for visual inspection of time-locked response of this single unit to voice waveform.

but was unaffected during evoked vocalization.

None of the inspiratory-modulated, group 4 single units (n = 5) showed RLN cooling effects. During evoked phonation, the single unit shown in Fig 8 was inspiratory-modulated. Peak activity followed the PGG waveform during vocal fold abduction (Fig 8). There was no RLN cooling difference and very little activity during phonation. Tonic activity (50 Hz) was seen during spontaneous respiration and was slightly augmented during inspiration.

Two of the group 5 single units (n = 7) showed changes after RLN cooling during spontaneous respiration and evoked vocalization, while 1 changed after cooling during spontaneous respiration only. Group 5 single units showed tonic or slightly phasic responses correlating with vocal fold movement as tracked by the PGG. In this group, there was a tendency to show tonic activity during evoked vocalization. One group 5 single unit showed a rhythmic variation during spontaneous respiration at 3 Hz that created a sawtooth pattern on top of a tonic rate of 25 to 30 Hz. During evoked phonation and before RLN cooling, the tonic rate increased by 10 to 15 Hz, but dropped off during inspiration when the vocal folds abducted (Fig 9). During the third phonation, another unexplained drop-off of activity occurred. After RLN cooling, the pattern seen during spontaneous respiration was not affected, but the single unit lost its rhythmic sawtooth pattern during evoked vocalization (Fig 9).

DISCUSSION

This study examined the effects of vocal fold paralysis on iSLN afferent activity. While loss of respiratory modulation of some iSLN receptors after RLN paralysis has been reported, effects of vocal fold paralysis on iSLN activity during vocalization had not been examined before. Many iSLN receptors are known to respond to vocal fold movement, muscle contraction and stretch, joint movement, and mucosal contact. We expected vocal fold paralysis to alter these behaviors and therefore predicted that many receptor response patterns would change during vocalization as a result of RLN cold block. Overall, the activity pattern changes seen due to RLN cold block were subtle. Only 24% of single units during spontaneous respiration and 18% during evoked phonation displayed activity pattern changes as a result of paresis. Even those single units that followed the respiratory movements of the vocal folds during spontaneous respiration usually maintained their response patterns when movements were eliminated by RLN cold block. This finding could be due to the depressive effects of the anesthetics used by others (see

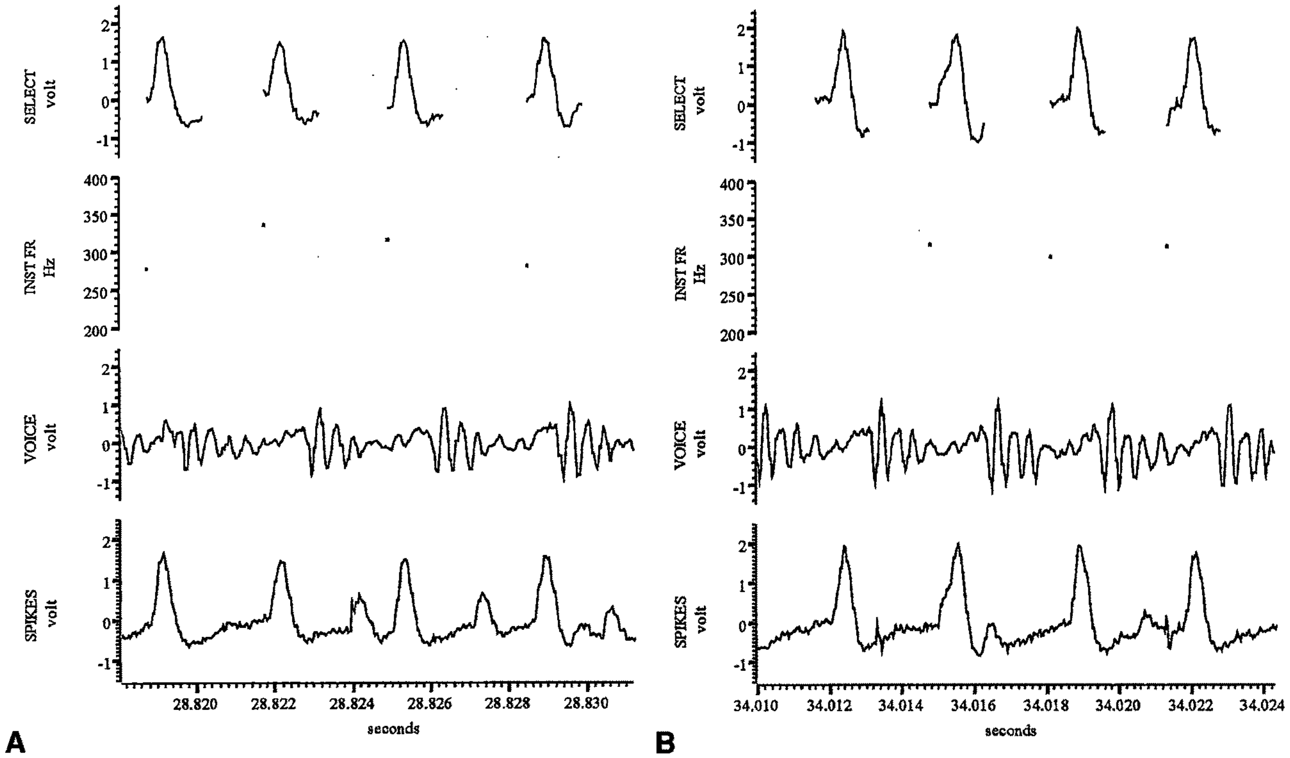

Fig 5. Visual inspection of time-locked following in group 2, frequency-following single unit. Note consistent alignment of spike peaks with voice waveform peaks both **A)** before RLN cooling and **B)** after RLN cooling. During portion of phonation shown, single unit is responding 1:1 to fundamental frequency of voice and vibration rate of vocal folds. Same single unit is shown in Fig 4.

below), as opposed to the vigorous respiratory and vocalization behaviors we saw with the decerebrate preparation.

Evidence from several prior studies led us to hypothesize that vocal fold paralysis by RLN cold block would produce a change in the responsiveness of laryngeal mechanoreceptors during respiration and vocalization.[2-4,14,15] Boushey et al,[2] in their study of the behavior of laryngeal afferents in cats, noted that paralysis and artificial ventilation were necessary to avoid additional stimulation by laryngeal motor responses. Sampson and Eyzaguirre[3] studied functional characteristics of mechanoreceptors in the larynx of cats and described mucosal "touch" and "deep" mechanoreceptors. The deep receptors were not activated by touch, but either by "displacement of the vocal cords or by compression and/or displacement of the outer walls of the larynx."[3(p465)] Both touch receptors and deep receptors commonly displayed respiratory modulation that was eliminated in every case tested[3] by RLN section or systemic paralysis with gallamine triethiodide. Electrical stimulation of the cut distal end of the RLN produced activation of both receptor types, especially when the receptors were located on or near the vocal fold itself. These authors stated that the discharge of touch receptors "is so readily modified by vocal cord movements and by vibratory stimuli that they may play a role in phonation."[3(p478)]

The work of Sant'Ambrogio et al[4] also supports the likelihood that some laryngeal receptors might be less active under conditions of vocal fold paralysis. They categorized laryngeal mechanoreceptors in dogs as pressure, flow, or "drive" receptors on the basis of their behavior during tracheostomy breathing, upper airway breathing, tracheal occlusion, and upper airway occlusion.[4] The use of this classification system was not possible in our study, since we were unable to perform the pressure manipulations because of the difficulty of maintaining a seal at the nose and mouth with a stereotaxic head holder and a fiberoptic scope in place. The current study was performed under different conditions in a different species and examined receptor activity changes during both respiration and vocalization. Nonetheless, some useful indirect comparisons can be made. Flow receptors are the least common receptor type, representing 14.6% of laryngeal receptors. Flow receptors would be unaffected by RLN cold block alone. The majority of receptors tested by Sant'Ambrogio et al[4] and Mathew et al[15] showed a predominant activity during inspiration, which was reduced after section of the RLN. We found that some respiratory-modulated afferents in the present study showed changes

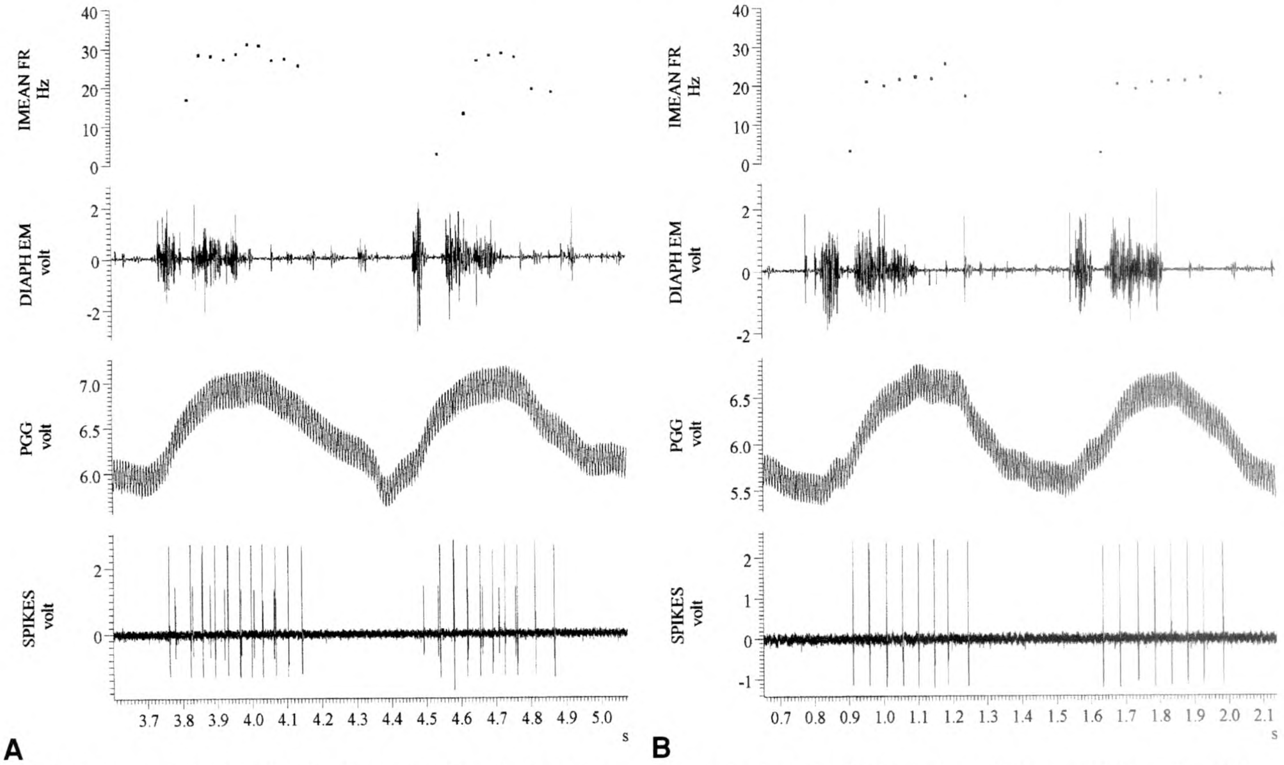

Fig 6. Group 3 single unit with change in firing during spontaneous respiration after RLN cooling. **A)** Before RLN cooling. **B)** After RLN cooling. Graph shows 20% drop in rate during phasic inspiratory phase spanning activation after cooling. See Fig 7 for evoked vocalization sequence of this single unit changed by RLN cooling.

in respiratory modulation after RLN cold block (Fig 6), but others did not (Fig 8). The number of afferents that showed a change after RLN cold block may have been lower in our study because of a lower incidence of inspiratory-modulated afferents identified. It is interesting to note that afferents that were inspiratory-modulated during spontaneous respiration could switch and become expiratory-modulated during evoked vocalization. This finding suggests the possibility of different roles for receptors that depend on the specific laryngeal behavior exhibited. Individual laryngeal mechanoreceptors are known to be multimodal in their responses, since they respond to a variety of both mechanical and chemical stimuli.[2,3,18,20]

Pressure receptors were the most common type found in the larynx by Sant'Ambrogio et al[4] and accounted for 63.6% of the total. Most of those pressure receptors responded to negative airway pressure, with only 11 of 70 responding to positive pressure. Mathew et al[14] confirmed the finding that negative pressure receptors are only minimally affected by positive pressure, but presented a figure showing the active response of a positive pressure receptor with both RLNs cut. Collapsing laryngeal pressure is said to be "the major stimulus in activating laryngeal afferents"[15(p41)] in anesthetized and spontaneously breathing dogs. With respect to positive pressure receptors, the majority (83%) have been said to be "low threshold," with the rest being recruited at higher positive pressure.[14] Some positive pressure receptors were undoubtedly activated during vocalization and were unaffected by RLN cold block in our study. It is not possible to predict how positive and negative pressure receptors behave during vocalization, especially when one considers the phase modulation switching described by us and the multimodal stimulus response characteristics[21] of laryngeal mechanoreceptors.

Some pressure receptors also show additional sensitivity to the respiratory movements of the upper airway and might be stimulated by the active muscle contractions that occur during vocalization. Mathew et al[14] reanalyzed a group of receptors that they initially thought were responding to pressure changes and found that 51% of the negative and 33% of the positive pressure receptors maintained a respiratory modulation even during tracheotomy breathing. That this "drive" component was attenuated or eliminated by paralysis confirmed its sensitivity to movement. Laryngeal "drive" receptors maintain their respiratory modulation during tracheotomy breathing, upper airway breathing, tracheal occlusion, and upper airway occlusion and seem to be stimulated by contraction of the laryngeal muscles in the absence of airflow and pressure in the larynx.[4] Each of the 5

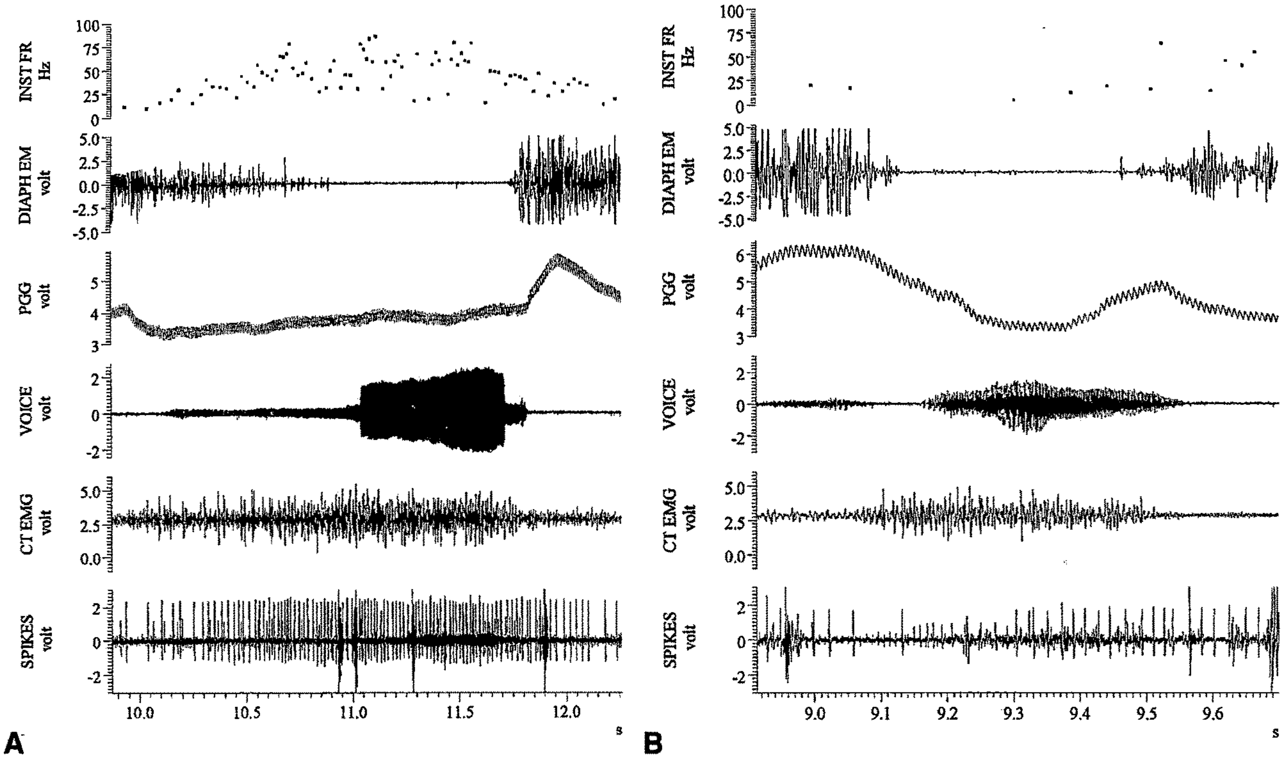

Fig 7. Group 3 single unit with change in firing during evoked vocalization after RLN cooling. **A)** Before RLN cooling. **B)** After RLN cooling. Graph shows significantly reduced activity. See Fig 6 for spontaneous respiration sequence of this single unit changed by RLN cooling.

"drive" receptors found by Sant'Ambrogio et al[4] shows a marked loss of activity during cold block of the RLNs.

We used a classification scheme that placed iSLN single-unit responses into groups based on the activity patterns seen during evoked vocalization. This allowed a comparison of the effects of paralysis between categories of afferent activity. Analysis of changes in response pattern following vocal fold paresis helped us understand more about the trigger stimuli for each group.

Group 1 single units responded maximally at the time of arytenoid closure during early phonation or just before phonation. This group had the highest percentage of single units affected by RLN cooling. Some aspect of vocal fold closure seemed to activate this group, since they were observed to respond whenever the arytenoid cartilages contacted each other, even when the animal did not phonate. Group 1 receptors may be located in the densely innervated vocal process area.[22] Vocal fold paralysis altered how tightly the vocal processes of the arytenoid cartilages were held together during phonation. This alteration could be responsible for the drop in activity seen by some of the group 1 single units.

Cold block–induced vocal fold paralysis always caused audible changes in vocal intensity, pitch, and quality during phonation. These changes were a reflection of an altered vocal fold vibratory pattern. Group 2 single units continued to track the vocal fold vibratory frequency pattern accurately after paralysis. Although a majority of laryngeal receptors are capable of following a mechanical oscillator,[3,17] group 2 receptors represent a specific vocal fold vibration–sensing group. We suggest that these receptors are positioned anatomically in such a way that the frequency-following characteristic is preserved. They may be located in a densely innervated zone along the medial edge of the thyroarytenoid muscle.[22] In this location they can respond to the glottic pressure wave or displacement of the edge of the membranous vocal fold without being stimulated by vocal process or arytenoid body contact during closure.

Group 3 single units responded to events occurring during phonation other than vibration or arytenoid contact. They might have responded to positive pressure, vocal fold tension or stretch, muscle contraction, joint movement, or epiglottic displacement. Group 3 receptor responses were expiratory-modulated and generally followed the envelope of the CT muscle EMG (Fig 7); therefore, tension generated from the contraction of that muscle could be a specific stimulus to these receptors.

All group 4 single units maintained inspiratory modulation during spontaneous respiration and evoked vocalization and were unaffected by RLN

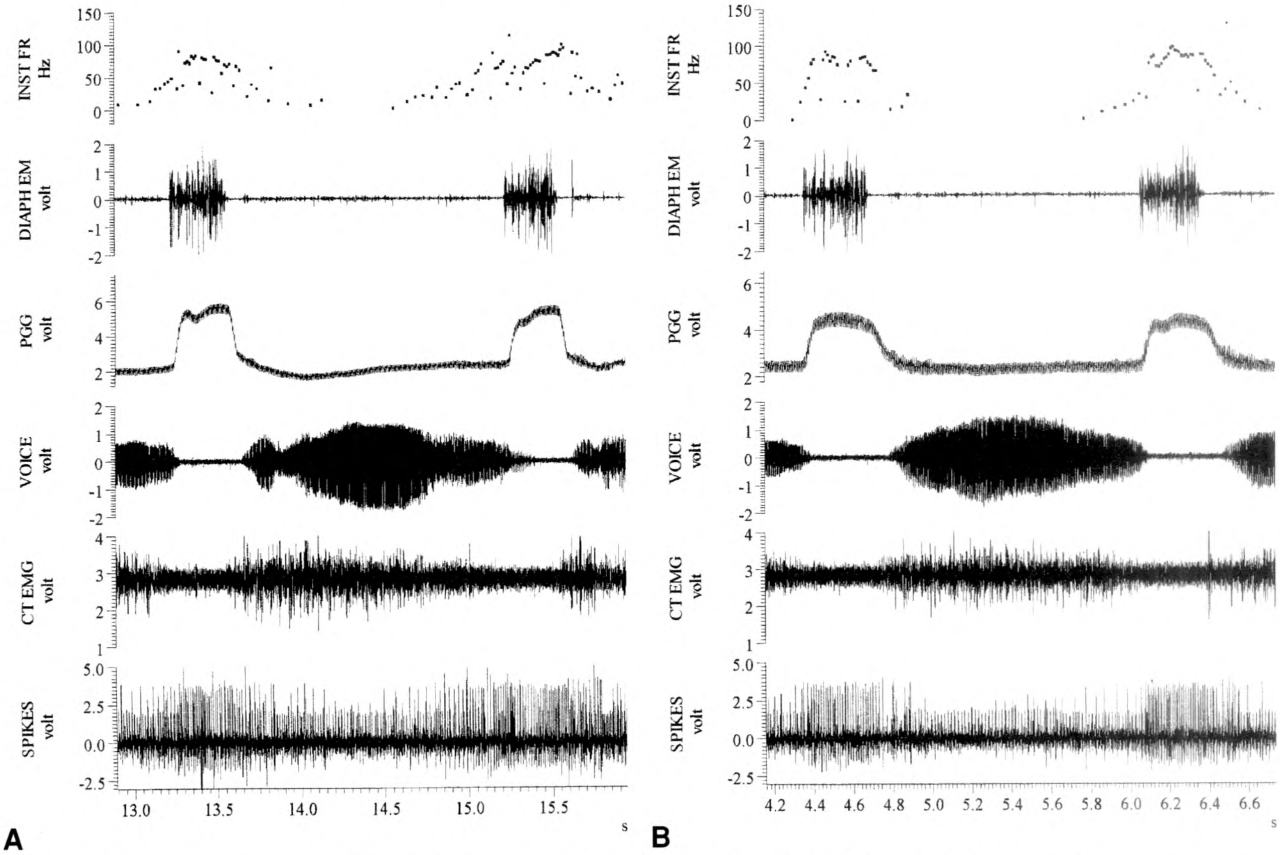

Fig 8. Group 4, inspiratory-modulated single unit unaffected by RLN cooling. During evoked phonation, this single unit showed peak activity that followed PGG waveform during vocal fold abduction with no RLN cooling difference. This single unit showed tonic activity (50 Hz) during spontaneous respiration that was slightly augmented during inspiration. **A**) Before RLN cooling. **B**) After RLN cooling.

cold block. In dogs, 65% of iSLN receptors are inspiratory-modulated during respiration, even when airflow is diverted from the larynx during tracheotomy breathing.[4] This finding suggests an effect due to the abducting action of the posterior cricoarytenoid (PCA) muscle, the only laryngeal muscle active during inspiration. Location of these receptors in the mucosa over the interarytenoid area or the PCA muscle itself[22] may explain their inspiratory modulation. Since these are midline structures, receptors located there would be able to respond to movements of the opposite PCA muscle and mobile vocal fold during abduction. Alternatively, group 4 receptors can respond to other stimuli, such as airflow or temperature,[4,13] that are little changed by paresis.

Group 5 afferents displayed the activity patterns that were the most varied during evoked vocalization. Five of 7 group 5 single units showed constant (tonic) activity during spontaneous respiration, with 4 showing phase modulation. These units may represent the "group 2" afferent fibers of Boushey et al,[2] which are slowly adapting and tonically active. These investigators used a systemic paralytic agent and mechanical ventilation and were therefore unable to evaluate respiratory phase modulation. It has been suggested that this type of receptor may participate in foreign body recognition.[17] Some of the group 5 receptors may be located in the supraglottis, and this location would explain the low incidence of RLN cold block effect. The unique example shown in Fig 9 seemed to respond to cardiovascular stimuli, as its sawtooth pattern followed the heart rate.

The position of many laryngeal receptors within the mucosa,[2,20,22] their ability to respond to multiple stimuli,[17] and the location of many of them near or on the arytenoid cartilages[17,23] may all be factors that allow them to continue to respond when the vocal folds are paralyzed. Mucosal receptors can continue to respond to mucosal contact, airflow, and displacement of laryngeal structures when the vocal folds become paralyzed. Muscle contraction and stretch, as well as displacement of laryngeal structures, presumably stimulate proprioceptive receptors within the muscles and tendons.[24] Vocal fold stretch and CT joint displacement through the action of the CT muscle might explain why some receptors responded in spite of RLN paralysis. We were unable to identify specific joint receptors in this study. Even though

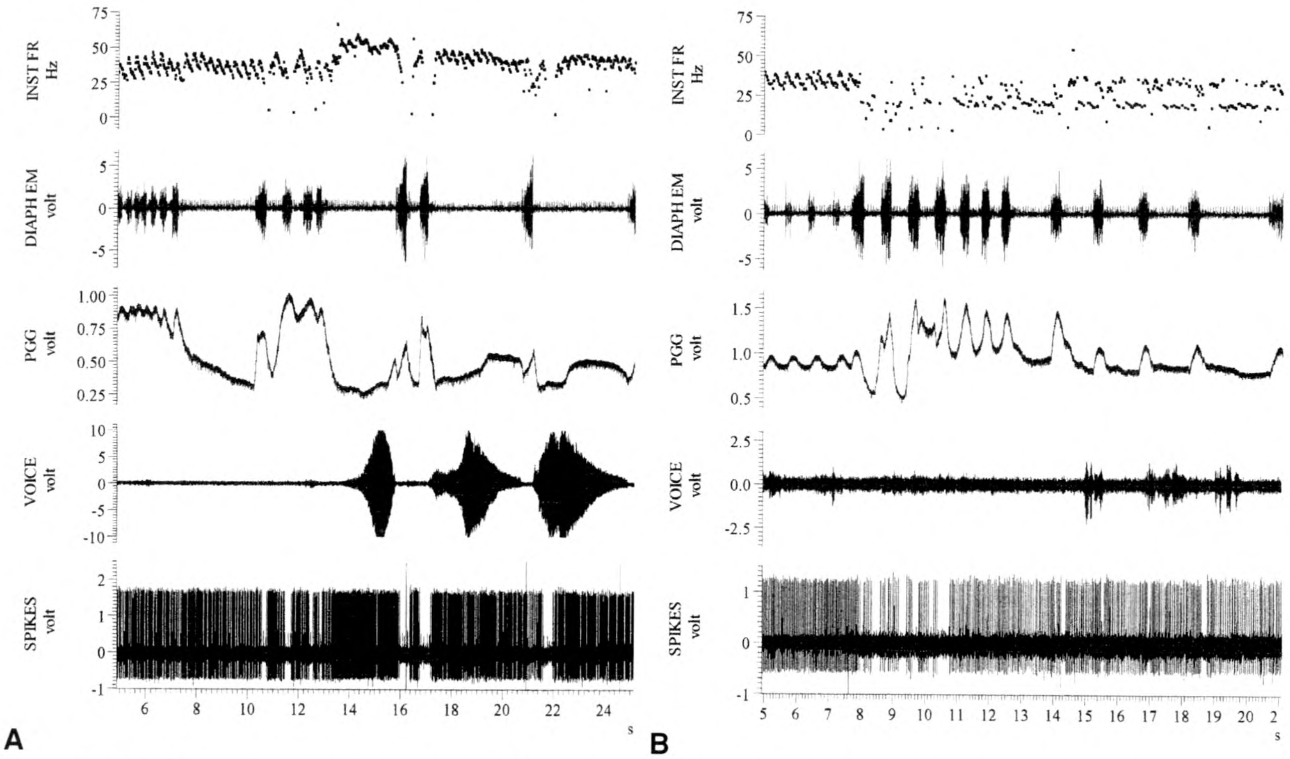

Fig 9. Group 5, miscellaneous single unit with RLN cooling effect and rhythmic variation during spontaneous respiration (1 to 7 seconds) at 3 Hz that created sawtooth pattern on top of tonic rate of 25 to 30 Hz. **A)** During evoked phonation before RLN cooling (8 to 25 seconds), tonic rate increased by 10 to 15 Hz, but it stopped during inspiration when vocal folds abducted. **B)** After RLN cooling, this group 5, miscellaneous single unit still showed sawtooth pattern during spontaneous respiration (1 to 8 seconds), but single unit did not maintain this pattern during evoked vocalization (8 to 21 seconds).

those receptors that responded to vocal fold vibration and closure did not change their basic response characteristics, their input was affected by differences in vocal fold vibratory pattern and quality of vocal fold apposition.

Data from this study showed that sensory information supplied to the central nervous system (CNS) by the iSLN remained largely intact during phonation when one vocal fold was paralyzed. In addition, a substantial proportion of that information was phonation-specific, and the CNS might use this sensory information to modulate vocalization. This use of sensory information is supportive of Wyke's[25] hypothesis of laryngeal prephonatory tuning and motor control during vocalization, which is based on afferent discharges from a "triad of receptor systems" including mucosal, muscle, and joint mechanoreceptors. On the other hand, investigators considering changes in laryngeal pressure and flow have supported functions of laryngeal receptors related to airway patency and defense.[26,27] They found it difficult to correlate receptor behavior with vocalization. Instead, the idea of central patterning of vocalization was proposed,[21] as well as control of vocalization via pulmonary receptors,[26] in lieu of input from laryngeal receptors.

We have detailed the effects of vocal fold paralysis on iSLN input during both respiration and vocalization. The classification scheme described served as a useful method of grouping iSLN behavior patterns. Consistencies in response existing among single units within a group before and after paralysis provide a basis for an improved understanding of laryngeal mechanoreceptor behavior. The relative insensitivity of iSLN activity to motor paresis suggests that the receptors studied are coupled to tissue such that passive interaction, rather than active muscle contraction, is the major stimulus. The CNS may use changes in afferent activity caused by altered vocal fold apposition and vibratory pattern to help compensate for the effects of vocal fold paralysis — but the central processing and utilization of this sensory input is not well understood.

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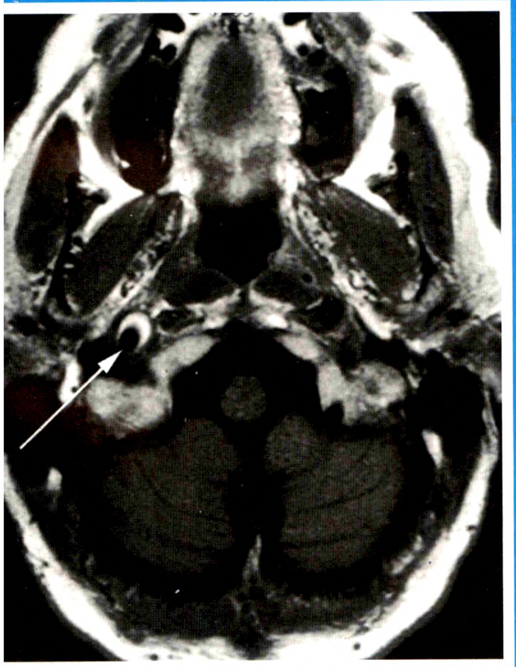

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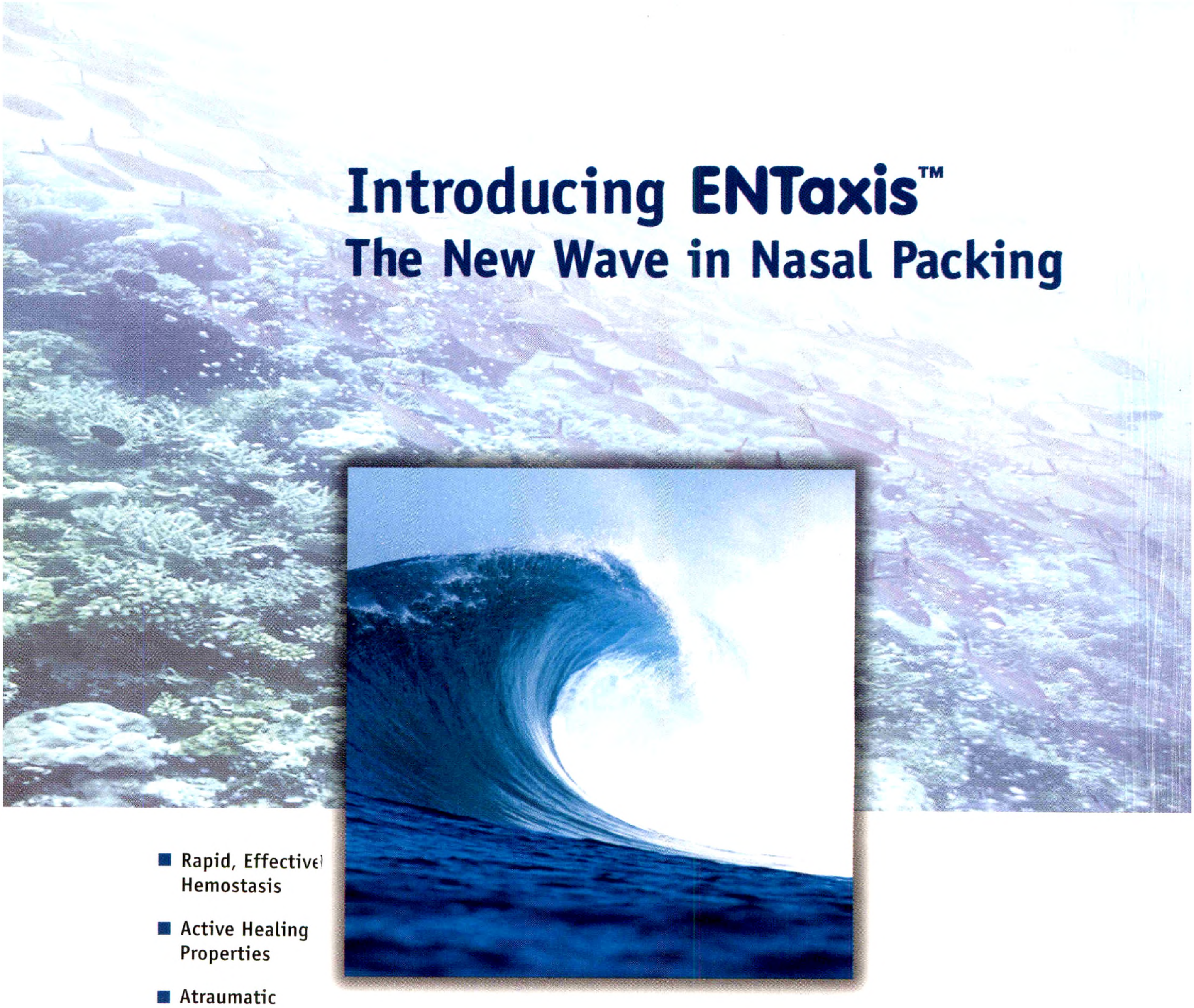

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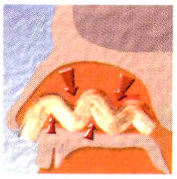

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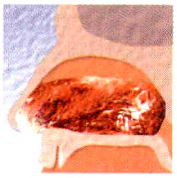

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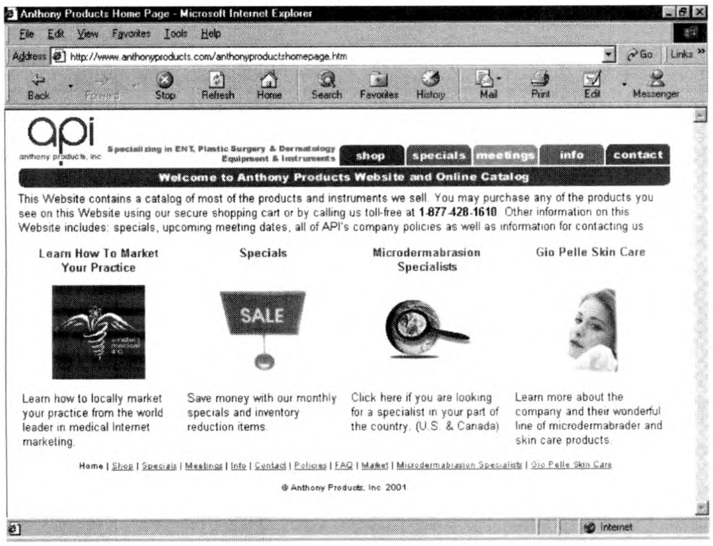

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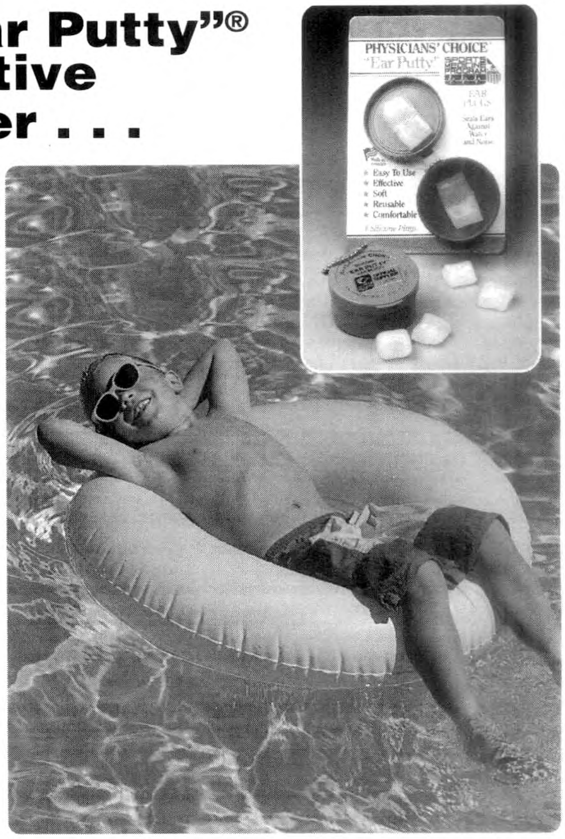

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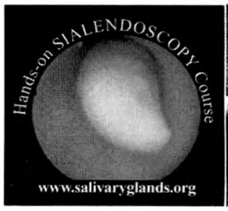

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CHANGING TRENDS IN ANGIOEDEMA

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Angioedema can be a life-threatening event presenting to otolaryngologists, emergency medicine physicians, and other physicians. Recent reports suggest an increasing role of angiotensin-converting enzyme inhibitors (ACEIs) in the causation of angioedema. Sixty-four cases occurring between 1994 and 1998 were identified and examined retrospectively. Fifty-eight percent of patients presenting with angioedema were using ACEIs. Ninety-four percent of patients overall, and 92% of patients with ACEI-associated angioedema, were African-American. Tongue, lip, facial, and supraglottic edema were most common. Treatment included intubation in 13% and intensive care unit monitoring in 20%. Nearly all patients were treated with corticosteroids and antihistamines. There were no deaths. Angioedema associated with ACEI use appears to be much more common than previously reported. African-American patients may be at higher risk for angioedema with ACEI use. Successful management was achieved with observation, expectant airway management, corticosteroids, and discontinuation of ACEIs. Patients without airway obstruction or pharyngeal or laryngeal edema who improved with treatment and observation were successfully treated as outpatients.

KEY WORDS — airway obstruction, angioedema, angioneurotic edema, angiotensin-converting enzyme inhibitors, laryngeal edema.

INTRODUCTION

Angioedema is a well-recognized clinical entity with various causes. The term is generally applied to patients presenting with edema, often involving the face, lips, tongue, pharynx, and larynx, that is not caused by infection, congestive heart failure, or other diseases that may cause swelling. Hereditary angioedema (HAE) accounts for a small percentage of cases. Adverse reactions to pharmacological substances, iodinated contrast dye, food, inhalants, and other substances have been implicated in sporadic forms. Idiopathic angioedema may account for a significant proportion of cases.[1] Angiotensin-converting enzyme inhibitors (ACEIs) have more recently been associated with angioedema. Each etiologic subtype causes the clinical entity of angioedema by different mechanisms. However, the end result of edema of the face and upper aerodigestive tract, with risk of acute upper airway obstruction, makes angioedema of any cause a concern for otolaryngologists, anesthesiologists, emergency medicine physicians, and any physicians participating in emergency airway management. Prior studies of patients presenting with angioedema have revealed an association with ACEI use in 24% to 35% of cases.[1,2] With widespread use of ACEIs in the management of hypertension and congestive heart failure, an increasingly larger percentage of patients presenting with angioedema seem to be concomitantly using ACEIs.

The aim of this study is to report a 5-year experience in treating patients with angioedema in a large, urban, university hospital with respect to epidemiology, etiology, airway management, medical treatment, and outcome.

MATERIALS AND METHODS

Patients were identified on a computerized database of ICD-9 (*International Classification of Diseases, Ninth Revision*) diagnostic codes for all patients seen in the emergency department or admitted to the hospital between January 1, 1994, and December 31, 1998. The presenting symptoms, physical signs including endoscopic examination, and hospital course were reviewed retrospectively. Ninety-four patients were initially identified with the database. Patients assigned a diagnosis of angioedema by an attending physician and exhibiting edema of the face, lips, oral cavity, oropharynx, hypopharynx, larynx, or other sites, without evidence of infectious, traumatic, or other clear causes, were considered as having angioedema. Those patients with clinical presentations inconsistent with angioedema, or who were given inaccurate ICD-9 codes, were excluded. In some cases, angioedema was listed in the medical record as an initial impression or as part of a differential diagnosis, and was incorrectly recorded as a diagnostic code. In other cases, patients with urticaria were incorrectly recorded as having angioedema. Sixty-two patients with 64 cases of angioedema meeting these criteria were identified and included in this study.

The racial makeup of patients admitted to the in-

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Presented as a poster at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

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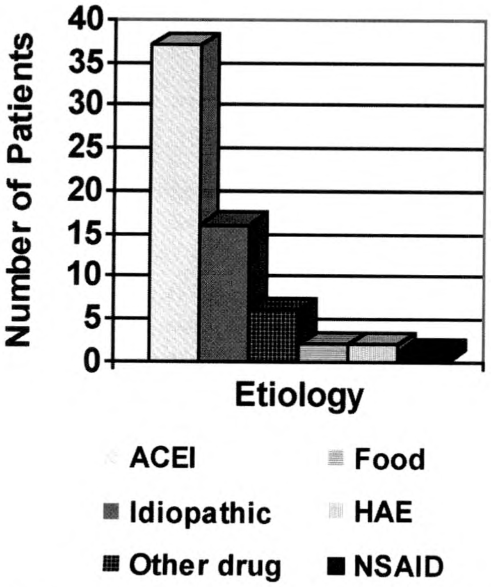

Fig 1. Presumed causes of angioedema. ACEI — angiotensin-converting enzyme inhibitor, HAE — hereditary angioedema, NSAID — nonsteroidal anti-inflammatory drug.

stitution during the study period was obtained from a computerized hospital database. The racial distribution of patients seen in the emergency department was obtained for the 1-year period from February 1, 1999, to January 31, 2000.

The χ^2 test was used for statistical analysis. Fisher's exact test was used when any variable value was less than 5.

RESULTS

Sixty-two patients presenting with angioedema were identified. The likely cause of angioedema was ascertained in 48 cases (75%) and remained unclear in 16 cases (25%).

Thirty-seven patients (58%) were using ACEIs. Sixteen of these patients had been using ACEIs for 3 months or less. Eight cases occurred within 2 weeks of therapy. In 7 cases, patients had been using ACEIs for 1 to 3 months. Another case began after a patient took an extra dose of ACEI after 2 to 3 months of therapy. Two additional cases occurred soon after a dosage increase after long-term therapy.

Six patients (9%) had angioedema after institution of other medications. Four episodes were temporally associated with the use of trimethoprim-sulfamethoxazole, chloramphenicol sodium succinate, clopidogrel bisulfate, and ranitidine hydrochloride, respectively. Another patient had angioedema while being treated with diazepam, temazepam, and nitroglycerin during a hospital admission. The sixth patient had recently started therapy with etodolac and yohimbine hydrochloride.

Two patients experienced angioedema after eating seafood and beets, respectively. Two patients presented a history consistent with HAE, although it was not confirmed by laboratory evaluation. Special serologic testing for C1 esterase inhibitor (C1EI) levels was not routinely performed in the acute care setting. One patient's presentation was consistent with nonsteroidal anti-inflammatory drug–related angioedema. The distribution of cases by presumed cause is summarized in Fig 1.

The patients' ages ranged from 31 to 92 years (mean, 56 years). The mean age of the patients using ACEIs was 58 years, compared to 54 years for those not using ACEIs. Overall, 39 patients (63%) were female and 23 (37%) were male. Among patients with ACEI-associated angioedema, 22 (59%) were female and 15 (41%) were male. These differences are not statistically significant.

Fifty-eight patients (94%) were African-American, while only 3 patients (5%) were white, and 1 patient (2%) was Hispanic. Thirty-four patients (92%) with ACEI-associated angioedema were African-American. In contrast, 50% of the patients admitted to this institution during the same time period were African-American, 33% were Hispanic, 14% were white, and fewer than 2% were Asian. The difference between the racial distribution of angioedema patients and that of all patients admitted is statistically significant ($p < .0001$). During a 1-year period, 73% of patients seen in the emergency department were African-American, 12% were Hispanic, and 8% were white. Hence, the difference between the racial distribution of angioedema patients and that of emergency department patients is also statistically significant ($p = .00126$).

The most common sites involved were the lip and tongue. Lip edema and tongue edema were present in 34 patients (53%) and 25 patients (39%), respectively. Facial edema was present in 13 cases (20%). Supraglottic edema was present in 9 cases (14%). Soft palate, tongue base, glottic, periorbital, extremity, and genital edemas were less commonly reported. The sites of involvement are summarized in Fig 2. Multiple sites of edema were seen in 29 cases (45%).

Among patients seen only in the emergency department, lip, tongue, and facial edemas were noted in 67%, 26%, and 21% of patients, respectively. Only 2 patients had edema of the oropharynx, hypopharynx, or larynx. Both patients had improvement noted at repeat endoscopy after a period of treatment and observation. No patient returned to the emergency department or was admitted with angioedema. The patients admitted to the hospital, however, had edema of the tongue, oropharynx, and larynx in 60%, 32%, and 32% of cases, respectively.

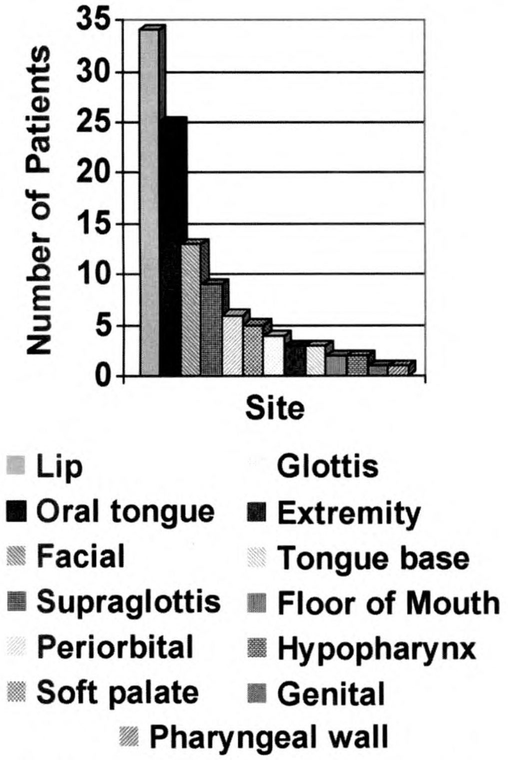

Fig 2. Anatomic sites of involvement of angioedema.

Eighteen patients (29%) reported prior similar episodes of swelling, including 6 patients (16%) with ACEI-associated angioedema. In 1 case, the patient had been using ACEIs at the time of the prior episode. One patient had 2 episodes of angioedema after discontinuation of ACEI use. Only 1 patient reported a family history of similar orofacial swelling.

Fifty-six patients (88%) presented to the emergency department, while 6 patients (9%) were already inpatients admitted for unrelated reasons. Twenty-eight percent of the patients seen in the emergency department and discharged were evaluated by an otolaryngologist. Seventeen of the 25 patients admitted (68%) were evaluated by the otolaryngology service as the primary admitting service or as consultants. Eight patients (13%) were intubated for airway protection. All intubations were performed by an otolaryngologist using awake, flexible fiberoptic bronchoscopic nasotracheal techniques. No tracheostomies or cricothyroidotomies were performed, and no deaths occurred. The patients who were intubated all had edema of the supraglottis and/or tongue. Overall, 6 of the 9 patients (67%) with supraglottic edema and 6 of the 25 patients (24%) with tongue edema were intubated. The duration of intubation ranged from 24 to 72 hours. No patients exhibited respiratory distress or stridor after extubation, and no patients were reintubated.

Thirteen patients (20%) were monitored in an intensive care unit, and 12 patients (19%) were observed in a medical or surgical inpatient unit. Thirty-nine patients (61%) were observed and discharged from the emergency department. Fifty-five patients (86%) received corticosteroids, and 61 patients (95%) received H1 blockers as part of their medical management. Three patients (5%) also received a subcutaneous epinephrine injection. Tongue edema was described in 2 of these cases, and stridor was described in the third case. None of these 3 patients were intubated. No patients were treated with antibiotics as part of their therapy for angioedema, and no patients developed any later signs or symptoms of infection.

The duration of medical therapy varied. In most patients admitted to the hospital, 24 to 48 hours of intravenous therapy was given. Dexamethasone sodium phosphate (10 mg every 6 to 8 hours) or methylprednisolone sodium succinate (40 to 60 mg every 6 hours after an initial dose of 125 mg) was used in most cases. This was often, but not always, followed by oral corticosteroids, ranging from a single dose to a 9-day taper. Patients treated solely in the emergency department generally received a single intravenous dose of methylprednisolone sodium succinate 125 mg, followed by a variable course of oral corticosteroids.

One patient was intubated after observation because of clinical and endoscopic evidence of progression after institution of medical therapy. No progression of symptoms or physical findings was documented in any other cases.

Use of ACEIs was discontinued in all cases, as was exposure to other likely etiologic agents in patients who were not being treated with ACEIs.

DISCUSSION

This study illustrates several important points regarding the changing etiologic trends in angioedema. The increasing predominance of ACEI use as a predisposing or causative factor, and the vast majority of cases in African-American patients in this series, are of considerable interest, given the widespread use of ACEIs in the treatment of hypertension and congestive heart failure.

Fifty-eight percent of patients in this series were using ACEIs for various periods of time before the onset of angioedema. This is a large increase in comparison to prior studies evaluating all cases of angioedema. Megerian et al[1] found that 35% of patients presenting with angioedema between 1985 and 1990 were using ACEIs. In contrast to this study, 59% of patients had no clear cause of angioedema. Only 25% of patients in this study had no identifiable inciting event or agent. Pigman and Scott[2] reported that 24%

of angioedema cases presenting to an emergency department between 1984 and 1991 were associated with ACEI use.

Ninety-four percent of the patients with ACEI-associated angioedema in this study were African-American. Only 3 white patients and 1 Hispanic patient presented with angioedema. This is significantly different from the racial distribution of patients admitted to this institution or seen in the emergency department. The higher incidence of hypertension[3,4] and congestive heart failure[5] in African-Americans than in white Americans may account for some of this difference. Brown et al,[6] however, reported a fivefold increased relative risk of angioedema in African-American patients compared to white patients in a report of ACEI-related angioedema. On the other hand, Thompson and Frable[7] found no racial predominance.

Most patients with angioedema in this study were female. Sixty-three percent overall, and 68% of patients with ACEI-associated angioedema, were female. Other studies, however, have not reported a sex predominance.[1,6-8] Male predominance has also been reported.[2,9]

Whether ACEI-associated angioedema is more or less severe than angioedema associated with other causes is unknown. Similar proportions of patients were admitted with ACEI-associated angioedema (35%) and with non–ACEI-associated angioedema (44%). Somewhat higher proportions of patients with ACEI-associated angioedema were admitted to the intensive care unit (27%) or intubated (16%) as compared to all other patients with angioedema (11% and 7%, respectively). These differences, however, are not statistically significant.

Brown et al[10] confirmed a much higher risk of recurrent angioedema in patients with continued use of ACEIs after a first episode of angioedema as compared to patients who discontinued their use. Continued use of ACEIs in that study may reflect less widespread knowledge of the association between ACEIs and angioedema during that study period, 1986 to 1992. In our current study, ACEI use was discontinued in all cases.

Sixteen percent of patients using ACEIs and 28% of patients overall in this study reported previous episodes of angioedema. These rates are similar to the findings of other studies.[6] A predisposition to angioedema with ACEI use among patients with a prior history of recurrent idiopathic angioedema was suggested by Orfan et al.[11]

Serologic testing for C1EI or other complement factors was not performed in the acute care setting for patients in this study. Only 2 patients had symptoms suggestive of HAE. One author reported normal serologic test results for C1EI, C4, and angiotensin-converting enzyme levels in a small group of patients with ACEI-associated angioedema.[12]

Early reports of ACEI-associated angioedema occurring after initiation of ACEI use appeared in the literature in the mid-1980s.[13] Slater et al[14] published a meta-analysis of 3 large studies regarding enalapril use. They reported a rate of 1 case per 3,000 patients during the first week of therapy, and a cumulative incidence of 0.1%. Two thirds of the "life-threatening" cases occurred during the first week of therapy. Although the majority of cases occurred after the first week of therapy, the rate of incidence after the first week was much lower. Angioedema was not dose-related in that study.

Hedner et al[8] reported 36 cases of ACEI-associated angioedema occurring in Sweden over a 10-year period. Forty-seven percent of patients had symptoms within the first week of treatment, and 77% within 3 weeks. Only 10 patients (28%) were hospitalized. The incidence of angioedema increased proportionally with ACEI sales during the study period.

Some authors have reported a higher risk of angioedema associated with lisinopril[6,9] and enalapril[6] as compared to captopril.

The mechanism of angioedema differs considerably among its many causes. Hereditary angioedema is caused by a decreased level of, or functionally defective, C1EI. The complement factor C1EI inhibits the activity of the first component of the complement cascade, as well as kallikrein, factor XI, plasmin, and activated Hageman factor. Its deficiency may increase activation of the complement cascade and vascular permeability, resulting in edema.[15] Local trauma may expose tissue factors that activate Hageman factor. Activated Hageman factor triggers clotting, kinin generation, and fibrinolysis. Fibrinolysis generates plasmin, which activates C1 and the complement cascade.[15]

The mechanism of ACEI-associated angioedema has been investigated in several reports. The ACEIs block the action of kininase II, which converts angiotensin I to angiotensin II, as well as inactivating bradykinin and other kinins. Bradykinin causes vasodilatation, increased vascular permeability, and histamine release.[16] Increased levels of bradykinin related to ACEI use may be responsible for angioedema. Increased wheal and flare reactions to subdermally injected bradykinin after ACEI ingestion have been reported.[17-19]

Allergic reactions to food, environmental contacts, insect stings, pharmaceuticals, and other substances may cause IgE-mediated immediate hypersensitivity, leading to mast cell degranulation. The resultant systemic release of histamine and lipid mediators may cause angioedema, either isolated or as part of a systemic anaphylactic reaction, within minutes after exposure. Also, IgE- and mast cell–mediated reactions leading to urticaria and angioedema can be caused by physical stimuli, eg, cold urticaria and exercise-related anaphylaxis.[20]

Iodinated radiocontrast media, opiates, highly cationic antibiotics, some muscle relaxants, and other agents can cause angioedema from nonimmunologic mast cell degranulation and histamine release. Nonsteroidal anti-inflammatory drugs may cause angioedema related to altered arachidonic acid metabolism. Serum sickness or necrotizing vasculitis may cause immune complex–mediated angioedema and urticaria.[20]

The best treatment for angioedema in general, and ACEI-associated angioedema in particular, has not been determined. Our approach to such patients has successfully avoided surgical intervention, morbidity, and, most important, mortality. Early fiberoptic examination of the airway identified patients with laryngeal edema or airway impingement by oral or pharyngeal structures. Awake fiberoptic nasotracheal intubation with the patient sitting upright and use of topical decongestion and topical anesthesia was performed by trained otolaryngologists. This method was successful in all cases attempted, and obviated the need for a surgical airway.

A controversial topic concerns which patients require hospital or even intensive care unit admission, and which patients can be safely discharged from the emergency department. The group of patients observed in the emergency department in this study had no upper airway obstructive symptoms. Only 2 patients (5%) had documented edema involving the oropharynx, hypopharynx, or larynx, and none had progression of edema during observation. Although no specific follow-up data are available, none of these patients was later admitted or again seen in the emergency department with angioedema. This outcome suggests that patients without upper airway obstruction, without edema of the pharynx or larynx, and who improve with treatment and observation can be relatively safely discharged from the emergency department.

Patients with edema of the pharynx, larynx, and tongue were frequently admitted for observation and, in some cases, were intubated for airway protection. Although some of these patients might have been safely discharged from the emergency department, this approach avoided morbidity and mortality related to airway obstruction.

Medical therapy with corticosteroids and antihistamines was used in nearly all cases. Progression of edema was seen in only 1 patient after institution of treatment. All intubated patients were successfully extubated within 72 hours.

Severe symptoms of IgE-mediated angioedema may be treated with subcutaneous epinephrine, and less-severe reactions are often treated with antihistamines.[21] Corticosteroids can be useful in preventing later recurrence of symptoms.[20]

The role of antihistamines in ACEI-associated angioedema is not clear. Although they were used in most cases in this study, the theoretical cause of the angioedema is increased bradykinin, and not histamine. Our experience using subcutaneous epinephrine was limited in this series of patients.

Exacerbation of HAE can be prevented with aminocaproic acid or tranexamic acid, both of which inhibit conversion of plasminogen to plasmin.[15] The synthetic "impeded" androgen, danazol, has been successfully used to prevent HAE attacks by increasing C1EI levels.[22] Short-term prophylaxis, for dental or other procedures, has been accomplished with fresh frozen plasma. Acute attacks are often treated with steroids, antihistamines, and/or epinephrine. Their efficacy, however, is not well established. Aminocaproic acid and androgens are not effective in acute care of angioedema.[15]

CONCLUSIONS

The majority of cases of angioedema in recent years have been associated with either short-term or prolonged use of ACEIs. African-American patients may be at higher risk of angioedema with ACEI use.

A combination of expectant airway management, close observation, corticosteroid administration, and ACEI cessation, when applicable, was associated with a successful outcome. That is, no surgical airways were required, there was no significant morbidity, and no deaths were seen. Patients without airway obstruction, who have no edema of the pharynx or larynx on examination, and who improve with treatment and observation can be successfully treated in an outpatient setting.

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Ann Otol Rhinol Laryngol 110:2001

COMPARISON OF MICROFLAP HEALING OUTCOMES WITH TRADITIONAL AND MICROSUTURING TECHNIQUES: INITIAL RESULTS IN A CANINE MODEL

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A prospective, blinded study was designed to determine whether placement of a microsuture in epithelial defects created on canine vocal folds results in histologically demonstrable improved healing. Epithelial defects similar to those created during microflap removal were made by means of traditional microsurgical techniques on both vocal folds of 4 adult dogs. One vocal fold defect on each dog was then closed with a single microsuture placed through the laryngoscope. The larynges were harvested approximately 6 weeks later, and blinded histologic quantification of scar formation was performed. Microsutures resulted in less scarring in all but 1 of the larynges studied. Unsutured vocal folds exhibited a 75% larger average scar cross-sectional area. Although the sample size was insufficient to establish statistical significance, the observed difference in scar formation between microsutured and unsutured vocal folds suggests that primary closure with a microsuture in the canine model results in less scarring than when healing occurs by secondary intention.

KEY WORDS — healing, histology, larynx, microsuture, phonosurgery.

INTRODUCTION

Benign laryngeal lesions are important causes of voice disorders and make up a large proportion of the typical laryngologist's practice. These lesions respond in varying degree to behavior modifications, including smoking cessation, adequate hydration, and the elimination of vocal abuse. A role for proton pump inhibitors and antireflux precautions has also been elucidated. Unfortunately, many of these lesions eventually require surgical intervention.

Excellent voice quality is dependent on the generation of a complex mucosal wave during phonation.[1] Vocal fold scarring disrupts this wave by causing a deficit in the superficial layer of the lamina propria with scarring of the vocal ligament (middle and deep layers of the lamina propria) to the overlying mucosa, resulting in suboptimal voice quality.[2] The patient often attempts to compensate for the resulting vocal deficit by changing the shape and position of the larynx with the extrinsic muscles, increasing the infraglottic air pressure, or changing the shape of the supraglottic resonators, resulting in worsened vocal dysfunction.[3] The importance of minimizing vocal fold scarring in order to allow the production of excellent phonation is therefore paramount.

Microsurgical treatment of laryngeal lesions is a relatively new technique. Few studies have evaluated vocal fold healing after microsurgical techniques. The use of microsuturing for the repair of microflap defects has only recently been reported, and its use does not appear to be widespread. This study is designed to show the efficacy of endoscopic microsuturing for the reapproximation of mucosal defects, and to present the technique to a wider population of practicing otolaryngologists.

MATERIALS AND METHODS

Four unrelated, heartworm-free, adult male mongrel dogs weighing between 18 and 27 kg (40 and 60 lb) were held in quarantine for at least 1 week. Animal care was supervised by The University of Texas Health Science Center at San Antonio (UTHSCSA) Laboratory Animal Resources Department follow-

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Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000. Presented in part at the Collegium Medicorum Theatri Conference, Aspen, Colorado, June 12, 1999.

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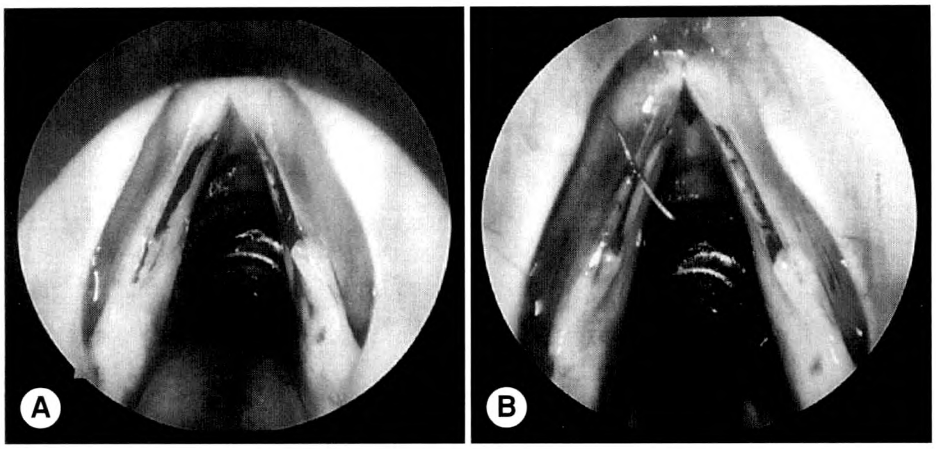

Fig 1. (Dog 2) Microflap epithelial defects. **A)** Symmetric defects consistent with those created by microflap techniques were made on each subject's true vocal folds. **B)** Alternating sides were closed with microsuturing technique.

ing a protocol approved by the UTHSCSA Institutional Animal Care Program.

No food or water was given for 12 hours before surgery. The dogs were premedicated with acepromazine maleate (5 mg subcutaneously) and anesthetized with intravenous sodium pentobarbital to a level of corneal sensation loss (approximately 10 to 20 mg/kg).[4,5] They were maintained at this level with halothane, ventilator-administered through a small-bore, cuffed endotracheal tube placed with its tip resting 5 cm below the vocal folds.[6] Direct laryngoscopy was performed to confirm normal laryngeal anatomy, and the laryngoscope was stabilized with a Lewy suspension apparatus. Photodocumentation was achieved with a Canon camera with a 90-mm lens modified for attachment to a 0° Hopkins telescope, model 8712AA (Karl Storz–Endoscopy America, Culver City, California).[7] The microflap defects were made in a manner consistent with currently used techniques[8] along the leading edge of the vocal fold at a depth to include the epithelium and sparing as much of the underlying lamina propria as possible. It should be noted that canine vocal folds do not have a well-defined vocal ligament; therefore, the flap elevation was kept immediately subepithelial, 1 to 2 mm lateral and medial-inferior to the defect margins. This flap involved surface dimensions of 4 to 7 mm in length and 2 to 3 mm in width (Fig 1). The vocalis muscle was preserved.[2] The anterior 3 to 5 mm of each vocal fold was not violated, as this has consistently been shown to result in anterior glottic scarring.[9]

On alternating sides from dog to dog, the microflap defect was closed with 1 microsuture. A microscopic flap elevator was used to undermine the epithelium 1 to 2 mm medial and lateral to the defect in a manner analogous to undermining skin edges for plastic closure of skin defects. Elevation was performed in the loose connective tissue layer immediately subepithelial with a blunt 30° elevator (Pilling Company, Fort Washington, Pennsylvania). Hemostasis was achieved with 1:10,000 epinephrine-soaked cottonoids and microsuction. A 6-0 double-armed fast-absorbing gut suture on a TG 140-8 needle (Ethicon Inc, Somerville, New Jersey) was loaded in micro–alligator forceps after removal of 1 needle. This suture is commonly used by ophthalmologists and is readily available. The suture knot was tied by a modification of a technique originally described by Woo et al[10] (Fig 2). The two ends of the suture were then cut with a microscissor. The vocal folds were sprayed with a premixed ampule of 4% lidocaine hydrochloride, and the laryngoscope was removed. The dogs were then awakened, extubated, and housed in the Laboratory Animal Resources Department, with arrangements made for adequate food and exercise.

Between postoperative days 39 and 49, each dog was euthanized with sodium pentobarbital after photodocumentation of the vocal folds was performed. A vertical midline incision was made in the neck, and the strap muscles were retracted laterally. The larynx was harvested from superior to the thyroid cartilage to the second tracheal ring. After fixation for several days in formalin, each larynx was split in the anteroposterior dimension. A sagittal section of each hemilarynx, including the section of the vocal fold that had undergone the microflap procedure and the adjacent supraglottic and subglottic structures, was created with a No. 15 blade. This sample was then cut in a coronal plane at the midpoint of the microflap defect. Mucosal biopsy specimens were fixed in hematoxylin and eosin. Masson's trichrome–stained slides were also made to allow a better description of the histologic architecture (Fig 3).[3,11]

A pathologist blinded to the specimen side that underwent microsuturing evaluated the resulting slides. Tissue from an unoperated canine vocal fold was also processed and compared to known normal canine vocal fold samples.[2,7,12-14] The defect sites were compared to prior investigators' findings regarding canine laryngeal wound healing patterns.[7,14-16]

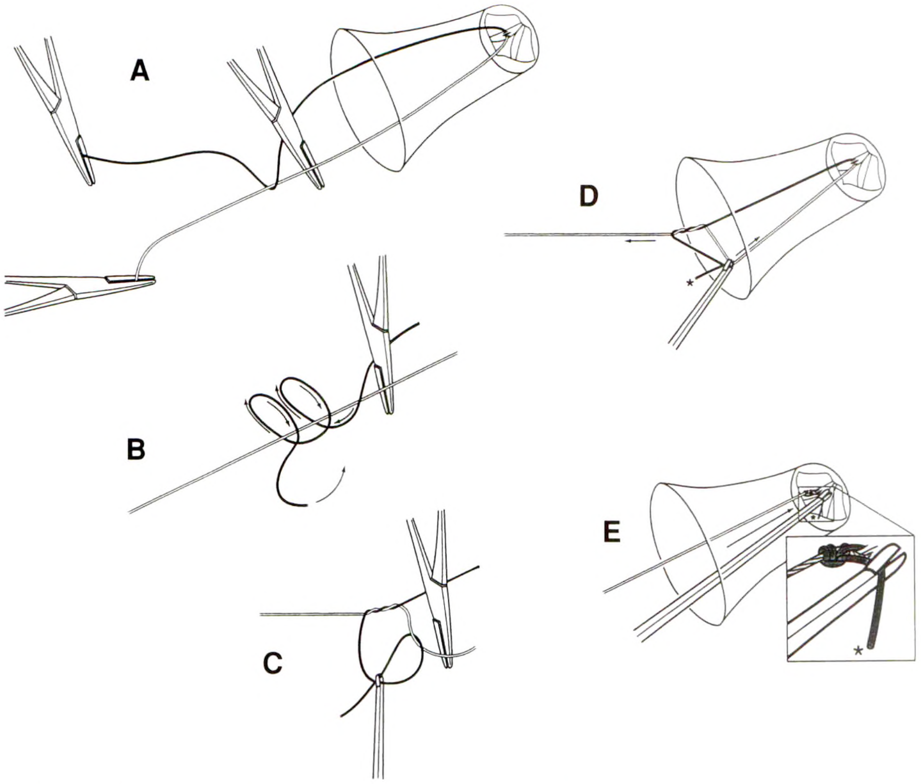

Fig 2. Endoscopic knot technique. **A)** Both suture ends are grasped with mosquito hemostats, and additional hemostat is placed between sutures distally, close to opening of laryngoscope. Black suture represents left side, and white suture is right. Left suture (black) is looped around right suture (white) in clockwise fashion. **B)** Left (black) suture is looped around right suture total of 3 times clockwise. Free end of left (black) suture is advanced toward initial (distal) crossing of two strands of suture. **C)** Free end of left (black) suture is used to create slipknot around open loop, adjacent to distal stationary hemostat, as shown. Micro–alligator forceps are used to grasp left (black) suture at its final "crossing"; this step prevents knot from forming in laryngoscope barrel and subsequently breaking. **D)** "Loose" knot assembly is advanced down laryngoscope by pushing distally with micro–alligator forceps and pulling back on other (white) suture. When level of true vocal folds is reached, one releases knot assembly with micro–alligator forceps, exercising care not to allow free end of left (black) suture (asterisk) to untie. Microscope can be brought back into field at this point. **E)** Free end of left (black) suture is grasped with micro–alligator forceps and advanced distally, while providing countertension on opposite (white) suture. Secure knot will form at level of vocal fold.

Quantitative evaluation of the extent of scarring was achieved for each slide by measuring the area of tissue involved with scar. Scar was identified as immature, plump, spindled to stellate fibroblasts with a loose myxoid collagenous stroma containing few elastic fibers. Each section was evaluated with a Zeiss microscope at 40 times magnification. A micrometer was used to determine the cross-sectional area of the scar in square millimeters. A percent difference in vocal fold healing between the two sides in each dog was calculated, and a paired *t*-test was performed.

RESULTS

The data for each dog are presented in the Table. Unsutured vocal folds exhibited a 75% larger average scar cross-sectional area than did microsutured vocal folds. The sample size was insufficient to establish statistical significance with a paired *t*-test, resulting in a p value of .092 with a 95% confidence interval of –0.027 to 0.207.

DISCUSSION

The myoelastic-aerodynamic theory of phonation was advanced by van den Berg[17] in 1958. This theory postulated that a complex interaction between airflow from the lungs and the mass and tension of the vocal folds produced vibration, resulting in voice production.[5] In 1975, Hirano[18] published his landmark description of the anatomy of the human vocal fold. He demonstrated that the vocal fold consisted of epithelium, the lamina propria, and the thyroarytenoid (or vocalis) muscle.[18] The lamina propria was further subdivided into superficial, middle, and deep layers. Most benign laryngeal lesions have been shown to involve only the superficial layer of the lamina propria (Reinke's space),[19] whereas fibroblasts necessary for the production of scar are primarily confined to the intermediate and deep layers of the lamina propria.[20]

Polyps, nodules, cysts, granulomas, and polypoid corditis (Reinke's edema) make up the majority of

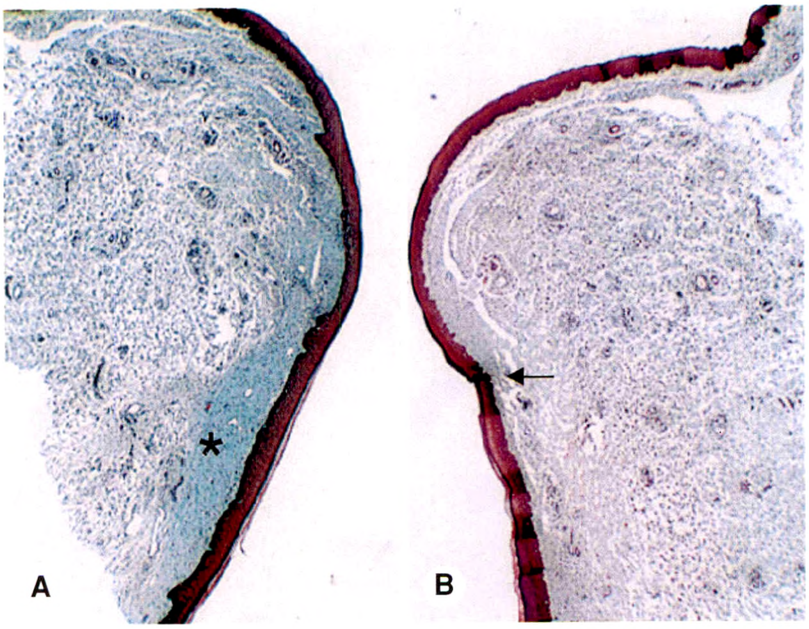

Fig 3. (Dog 3) Comparative regions of mucosal scarring (Masson's trichrome stain, original ×20). Representative cross sections of region of scar for **A)** unsutured vocal fold (asterisk) and **B)** sutured vocal fold (arrow). Note relatively smaller area of scar on sutured vocal fold. Scar tissue was differentiated from native connective tissue and conus elasticus by means of high-magnification evaluation of histomorphology with tri-elastic histochemical stain. Scar tissue was composed of immature, plump, spindled to stellate fibroblasts with loose myxoid collagenous stroma containing few elastic fibers. In contrast, native connective tissue and conus elasticus were composed of mature spindled fibroblasts with more densely collagenous stroma containing numerous elastic fibers. These differentiating features are not apparent in these low-magnification photomicrographs.

benign laryngeal lesions. Through the mid-1970s, vocal fold "stripping" was the most accepted method for treating benign laryngeal lesions.[20] This involved the removal of the vocal fold mucosa sometimes down as deep as the vocal ligament or even the muscular layer, resulting in a fibroblastic response, significant vocal fold scarring, and disruption of the mucosal wave that could result in a poor vocal outcome.[2]

Current surgical techniques emphasize the preservation of the middle and deep layers of the lamina propria (the vocal ligament) by approaching lesions through the superficial layer of the lamina propria via a lateral or medial microflap, as described by Bouchayer and Cornut[21] in France and Courey et al[19] in the United States. The goal of this technique is restoration of normal vocal fold anatomy and histology with minimal disruption of the normal surrounding structures.[19] When lesions are appropriately selected, excellent results in terms of postoperative voice and vocal fold vibratory characteristics can be obtained. Blinded perceptual analysis of paired voice samples obtained before and after medial microflap surgery by Courey et al[8] revealed improvement in 16 of 17 patients. All of the patients discussed in that study showed maintenance or return of the mucosal wave, and 15 of the 17 had improved glottic closure patterns. An earlier study by Courey et al[19] found that 28 of 30 patients who underwent microflap procedures rated themselves as clinically improved.

SCAR CROSS-SECTIONAL AREA

	Dog 1	*Dog 2*	*Dog 3*	*Dog 4*	*Average*
Sutured fold (mm^2)	0.17	0.05	0.12	0.16	0.12
Unsutured fold (mm^2)	0.24	0.17	0.30	0.16	0.22
Absolute difference (mm^2)	0.07	0.12	0.18	0.00	0.09
Percentage difference	41	240	150	0	75

When recurrent or scarred lesions and large benign vocal fold lesions are removed with a microflap technique, significant mucosal defects may result. Current practice is to allow these defects to granulate and heal by epithelial migration.[10] This can result in a nonvibratory segment of the vocal fold that prevents propagation of the mucosal wave, causing aperiodicity of the glottal sound.[10] In 1995, Woo et al[10] suggested that the placement of a microsuture would result in improved wound healing by allowing primary healing and reducing tension on the wound site during the healing process. They also proposed that a microsuture could allow the surgeon to position the scar away from the vocal fold edge, resulting in less trauma to the wound. In a series of 18 patients who underwent this procedure, Woo et al reported excellent results, with all patients reporting a subjective improvement in their voices. Videostroboscopic evaluation of these patients showed consistent improvement in mucosal wave, amplitude and vibratory function. Unfortunately, the study of Woo et al was not controlled, and no comparison can be made to standard microflap techniques.

Because of the relative inaccessibility and indispensable nature of the human larynx, the development of an easy-to-use, functionally representative, and generally accepted model of this organ is important. Leonardo da Vinci was the first to record attempts to examine the source of the human voice using excised cadaver larynges.[5] Others have used this model also, but basic postmortem changes in vocal fold vibratory properties[22] and muscular tension[23] have been extensively documented, resulting in its limited use. Mathematic modeling has allowed description of basic vocal fold vibratory properties, but remains limited in its ability to characterize voice production.[5]

Ferrien was the first to use a canine model, in 1741, when he used canine larynges to show that vocal intensity was influenced by glottal width and air velocity.[5] Since then, this model has become the most accepted experimental model of the human larynx. The canine larynx is similar in size and histology to the human larynx, but exhibits a thicker lamina propria. It vibrates on a 2-mass system, as does the human larynx. A critical difference between human and canine vocal folds is the fact that in dogs, there is no well-defined vocal ligament.[5] Because of this key difference, one must interpret canine data with caution. These differences in the lamina propria can have important implications that limit the clinical utility of canine vocal fold studies. Canine vocal fold vibratory characteristics in vivo are nonetheless very similar to those found in humans, so they have been studied extensively.[5,12] Canine larynges also have been found to exhibit many pathological conditions similar to those found in humans, including squamous cell carcinoma and polyps.[24,25] Finally, canine vocal fold healing has been shown to be similar to human vocal fold healing.[7]

Traditional evaluation of epithelial tissue is accomplished by hematoxylin and eosin staining, but this technique is often deficient in evaluating vocal fold healing because of extensive artifact. Van Gieson and Masson's trichrome stains of connective tissue allow a better description of the histologic architecture.[3,11] Standardized scoring systems have been proposed for characterizing gross and histologic findings for injured vocal folds,[7] and vocal fold scar quantification techniques have been developed by Coleman et al.[26]

The purpose of developing microlaryngeal techniques is to minimize surgically induced trauma to the delicate microstructure of the vocal folds. The microsuturing technique presented here provides a straightforward technique based on sound wound healing principles to reduce vocal fold scarring and improve phonation. The main disadvantage to this technique is the time involved in placing the microsuture. A learning curve is encountered, but it can be partially alleviated by practicing the technique outside the operating room with larger suture before attempting it intraoperatively. With practice, a suture can be passed, tied, and tightened in less than 5 minutes consistently.

In our series, 3 of the 4 canine larynges studied demonstrated less scarring when epithelial defects were closed primarily with a microsuture instead of being left to heal by secondary intention. In the fourth larynx, there was no difference between the sutured and unsutured sides. While these data did not achieve statistical significance ($p = .092$), they do suggest a relationship between microsuturing and improved healing. Future efforts may achieve statistical significance through the use of an increased sample size.

CONCLUSION

These data suggest that vocal fold epithelial defects in a canine model demonstrate less scarring when primary closure with a microsuture is performed than when healing occurs by secondary intention. Although the sample size was insufficient to establish statistical significance, there was an observed difference in quantified scar formation between microsutured and unsutured vocal folds of 0.12 mm^2 and 0.21 mm^2, respectively ($p = .092$, 95% confidence interval of –0.027 to 0.207). This preliminary evidence suggests that microsuture closure of epithelial defects of the vocal folds may decrease scarring, at least in a canine model. Further research is warranted to determine whether suturing will improve wound healing, phonatory quality, and mucosal wave characteristics in human subjects.

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AVOIDING INTUBATION IN THE INJURED SUBGLOTTIS: THE ROLE OF HELIOX THERAPY

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Intubation in the child presenting with severe viral tracheobronchitis or prior subglottic injury can be detrimental to the child and the subglottis. Intubation may lead to further mucosal ischemia, scar, subglottic stenosis, or failed extubation requiring a tracheotomy. Heliox is a combination of helium and oxygen that produces less-dense gas exchange. Its use leads to a decrease in turbulent airflow, which may obviate the need for intubation. Here we report our experience using heliox as initial therapy in 14 consecutive children presenting with severe airway distress and the need for intubation. (Five had viral tracheobronchitis, 5 had inflammatory exacerbation of subglottic stenosis, and 4 had acute iatrogenic subglottic injury.) In 10 of the 14 children, intubation, which can lead to mucosal injury and scarring, was avoided by the use of heliox therapy. Of the 4 children in whom heliox therapy failed, 3 had a prior history of subglottic stenosis. Heliox is a relatively safe and reliable alternative to intubation of children with severe subglottic edema or injury. Heliox should be considered before intubation for selected children with subglottic inflammation and severe airway distress.

KEY WORDS — heliox, subglottic stenosis, viral tracheobronchitis.

INTRODUCTION

Heliox is a gas mixture composed of helium and oxygen. Its use in upper airway obstruction was first suggested in 1934.[1] In spite of its introduction more than 60 years ago, it has never seen widespread clinical use. Heliox has a density one third that of room air, and it is this property that makes it useful in the treatment of airway obstruction. Heliox decreases resistance to flow, changes turbulent flow to laminar flow, and promotes the diffusion of carbon dioxide and thereby decreases the work of breathing.[2-7] Ultimately, these advantages may obviate the need for intubation. Intubation can be detrimental to the upper airway, including the subglottis, where it may lead to further mucosal injury or scar.[8] We present 14 consecutive pediatric patients who developed severe respiratory distress that failed to resolve with conventional medical treatment who were subsequently treated with heliox. Our goal was to determine whether endotracheal intubation could be avoided through the use of heliox.

MATERIAL AND METHODS

Patients who had presented to Wake Forest University Baptist Medical Center–Brenner Children's Hospital in severe respiratory distress due to an upper airway lesion were initially treated with conventional medical therapy consisting of humidified oxygen, intravenous steroids, and nebulized racemic epinephrine bitartrate. Heliox was used in 14 consecutive patients in whom this treatment failed and in whom intubation or reintubation was impending. Five of the patients had a primary diagnosis of viral tracheobronchitis, 5 had an inflammatory exacerbation of underlying subglottic stenosis, and 4 had an acute traumatic injury to the subglottis. All of the patients were initially treated with commercially prepared heliox composed of 70% helium and 30% oxygen. Although heliox does not have approval from the US Food and Drug Administration for this indication, it has been used in our institution without any side effects. The heliox was delivered by either an oxyhood or a non-rebreather face mask, or through a continuous positive pressure mask. In children in whom heliox treatment was successful, the patient was subsequently weaned to humidified oxygen without helium.

RESULTS

We present 2 representative case reports, followed by a summary of our 14 cases.

Case 1. A 4-month-old girl presented to an outside hospital with a 5-day history of noisy breathing. The diagnosis made was bronchiolitis. She was subsequently released and referred as an outpatient to our institution. The patient's medical history was significant for intrauterine growth retardation, as well as respiratory distress at birth that required intuba-

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Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, April 26-27, 1999.

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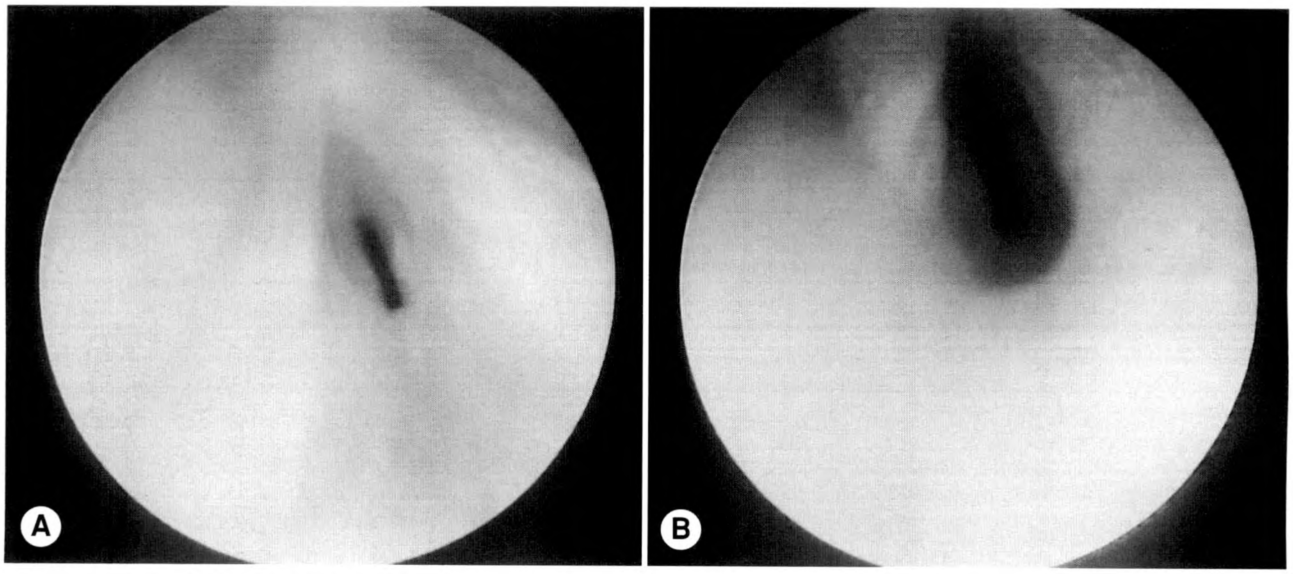

Fig 1. (Case 1) Findings at endoscopy. **A)** Glottis and subglottis. **B)** Closer view of subglottis.

tion. The patient's initial examination revealed biphasic stridor and mild retractions. A further review of the history revealed a choking spell prior to the onset of the noisy breathing. In view of the possibility of foreign body aspiration, it was decided to take the patient to the operating room, where she underwent direct laryngoscopy and bronchoscopy. This procedure revealed severe subcordal edema that extended into the subglottis (Fig 1). On the first postoperative day, the patient developed respiratory distress requiring intubation. It was believed that the patient's respiratory distress was due to postbronchoscopy edema on top of the existing subglottic edema. After extubation, the patient developed respiratory distress that failed to resolve with intravenous steroids, racemic epinephrine, and humidified oxygen. The patient was placed on heliox via a non-rebreather mask, which she tolerated well. The patient remained on heliox for a period of 24 hours, after which time she was successfully weaned to room air.

Case 2. A 2-month-old boy with a 2-day history of stridor, croupy cough, and fever to 41°C (106°F) presented to an outside hospital emergency department. On arrival, he developed cardiopulmonary arrest. The patient was intubated with an uncuffed 4.5-mm (internal diameter) endotracheal tube, resuscitated, and transferred to our institution for further evaluation and treatment. Examination in the pediatric intensive care unit revealed blood in the endotracheal tube suggestive of traumatic intubation. The patient's endotracheal tube was immediately downsized to a 3.0-mm (internal diameter) tube. The patient was taken to the operating room, where he underwent direct laryngoscopy and bronchoscopy that revealed extensive subglottic edema and mucosal ischemia (Fig 2). He remained intubated for 9 days while his medical condition stabilized. After extuba-

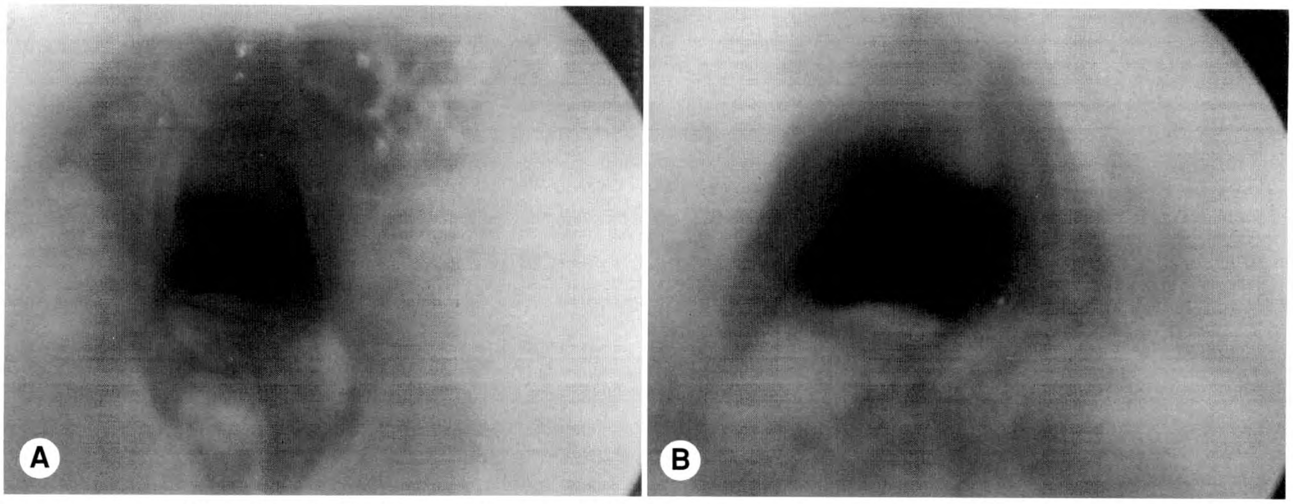

Fig 2. (Case 2) Findings at endoscopy. **A)** Glottis and subglottis. **B)** Closer view of subglottis.

PATIENT DATA

Age	Airway Diagnosis	Route of Heliox Delivery	Duration of Heliox Use	Intubation Avoided
4 mo	Subglottic stenosis	Oxyhood	24 h/6 d*	On second attempt
1 mo	Subglottic stenosis	Oxyhood	4 d	Yes
3 y	Tracheo-bronchitis	CPAP	2 d	Yes
2 y	Traumatic injury	Face mask	4 d	Yes
7 mo	Traumatic injury	Oxyhood	36 h	Yes
2 mo	Subglottic stenosis	Oxyhood/ CPAP	36 h	No
2 mo	Tracheo-bronchitis	Oxyhood	4 d	Yes
1 mo	Subglottic stenosis	CPAP	3 d	Yes
3 mo	Traumatic injury	Face mask	36 h	Yes
1 mo	Tracheo-bronchitis	Oxyhood	4 d	Yes
2 y	Tracheo-bronchitis	Face mask	36 h/12h*	On second attempt
2 mo	Traumatic injury	Oxyhood	36 h	Yes
3.5 mo	Subglottic stenosis	Face mask	24 h	No
2 y	Tracheo-bronchitis	Face mask	20 h	Yes

CPAP — continuous positive airway pressure.

*For patients in whom heliox therapy initially failed but who were subsequently extubated by means of heliox, both treatment intervals are given.

tion, he developed respiratory distress that failed to resolve with medical treatment. Heliox was successfully instituted, and reintubation was avoided. The patient was successfully weaned to room air after 36 hours of treatment.

Of the 14 patients in whom heliox therapy was implemented, intubation or reintubation was avoided in 10 (see Table). Of the 4 patients who required airway intervention, 2 were subsequently successfully extubated with the use of heliox. The duration of heliox therapy ranged from 20 hours to 6 days. In several patients, as the heliox was discontinued, the stridor and respiratory distress returned, requiring reinitiation of the heliox therapy. In these patients, once the underlying upper airway disorder improved, heliox was successfully discontinued. In the 2 patients in whom the heliox was ultimately unsuccessful, there was evidence of tongue base obstruction. Three of the 4 patients in whom heliox therapy initially failed had severe underlying subglottic stenosis, and 2 of them eventually required tracheotomy.

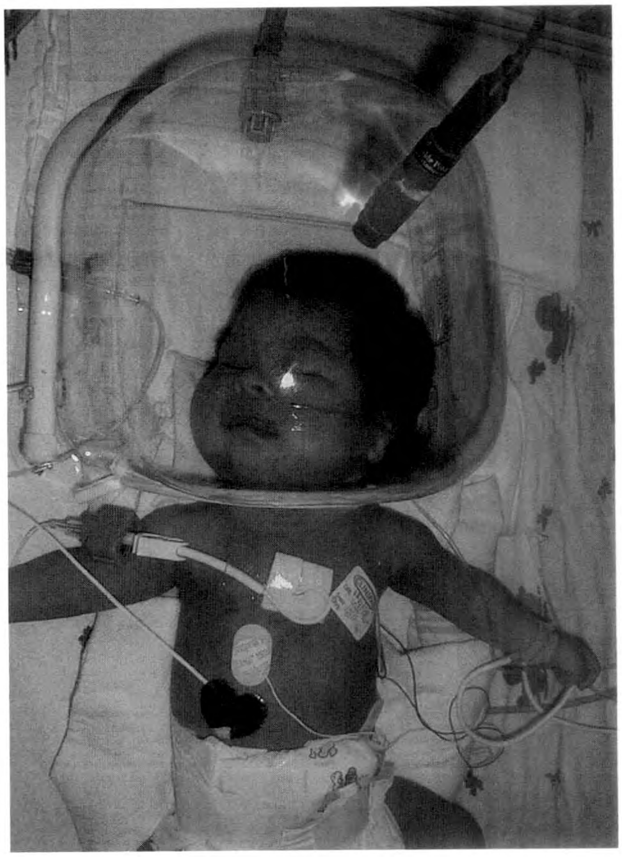

Fig 3. Patient undergoing heliox therapy in which gas mixture is delivered via oxyhood.

DISCUSSION

The use of heliox in airway obstruction was first described by Barach[1] in 1934. Its use was limited by military demand during World War II, and it never gained widespread use.[9,10] Heliox is available commercially in concentrations of 70% to 80% helium combined with 20% or 30% oxygen. Additional oxygen can be added from a separate source. However, as the concentration of helium is decreased, it may lose its advantageous properties.[11] It can be delivered through a non-rebreather face mask or a continuous positive airway pressure mask, or via an oxyhood (Fig 3).

Heliox has been used in multiple clinical settings. The literature includes case reports of its use in respiratory distress syndrome, postextubation stridor, asthma, chronic obstructive pulmonary disease, croup, bronchiolitis, and primary airway tumors.[4,5,12-16] It has also been used to facilitate ventilation during flexible bronchoscopy, in cases in which the bronchoscope is close to the diameter of the endotracheal tube it is inserted through.[17] Interestingly, despite its particular applicability to upper airway obstruction, an extensive literature search revealed no case reports in the otolaryngology literature.

Heliox has multiple advantages over room air and oxygen. It decreases the resistance to airflow, converts turbulent flow to laminar flow, increases the diffusion of carbon dioxide, decreases the work of breathing, and ultimately may obviate the need for intubation.[2-7] These advantages are derived from the fact that helium has a density one seventh that of nitrogen, and heliox has a density one third that of room air.

As previously stated, heliox converts turbulent flow to laminar flow. It takes substantially less pressure to drive a gas under conditions of laminar flow than it does under turbulent flow. Whether flow will be turbulent or laminar is determined by Reynolds' number. Reynolds' number is calculated from the formula $Re = 2rvd/\eta$ where Re = Reynolds' number, r = radius, v = velocity, d = density, and η = viscosity. Reynolds' numbers of less than 2,000 are associated with laminar flow. Gases with lower densities will have a lower Reynolds' number and will be more likely to course through the airway under conditions of laminar flow.

In cases in which flow remains turbulent, heliox continues to have a beneficial role in decreasing resistance. During conditions of turbulent flow, the driving pressure is directly proportional to the density of the gas and inversely proportional to the fifth power of the radius. Although resistance during turbulent flow varies depending on the rate of flow, it is directly related to the density of the gas. Experiments that have examined the decrease in resistance to flow of heliox relative to room air report a 20% to 40% reduction in resistance, depending on the respiratory rate and the disease process in question.[3,6,7]

Heliox also increases the diffusion of carbon dioxide. Carbon dioxide diffuses 4 to 5 times faster in heliox than in room air.[18] In patients with hypercarbic respiratory failure, this offers a significant advantage. The decrease in the partial pressure of carbon dioxide may be associated with an improvement in respiratory acidosis, a quality that has also been ascribed to heliox.[17-20]

Heliox has also been found to decrease the work of breathing. In 1983, Skrinskas et al[4] determined that breathing heliox at a respiratory rate of between 24 and 36 breaths per minute was associated with a 31% to 36% decrease in the work of breathing. This decreased work of breathing also results in decreased carbon dioxide production and oxygen consumption.[5] In pediatric patients, the trachea is particularly pliable. The decreased intrathoracic pressures generated during heliox use may be associated with decreased tracheal collapse and less dynamic narrowing of the airway.[15]

Ultimately, heliox may obviate the need for intubation. In 4 of the patients in our series, intubation trauma was the primary cause of the airway distress. In 3 additional patients, it may have been a contributing factor in the increase in subglottic inflammation. Intubation may also lead to further mucosal ischemia, injury, scar, and subglottic stenosis.[8] In this study, 10 of the 14 patients did not require intubation after the institution of heliox therapy. Of the 4 remaining patients, 2 were subsequently extubated with the help of heliox. Of the 4 patients in whom heliox therapy initially failed, 3 had significant underlying subglottic stenosis. The 2 patients in whom subsequent extubation attempts failed had tongue base obstruction, which contributed to their airway distress.

Heliox is an inert gas that is near-insoluble in tissue and is nonreactive with biological membranes. As a result, there are few complications reported in the literature. In our series of 14 patients, there were no observed side effects. Two reported side effects are hypothermia and hypoxemia.[12,21] The hypothermia relates to the thermal conductivity of helium, which is 4 to 6 times greater than that of nitrogen.[10] Warming of the inspired gas mixture should prevent this potential complication from occurring. Hypoxemia may be related to the diminishing benefit of heliox as the oxygen content is increased and the helium content is decreased.[11]

CONCLUSIONS

Heliox may be an important adjunct to the medical treatment of upper airway obstruction. It may serve as a temporizing agent to allow for the natural resolution of the disease process. The use of heliox should be considered selectively in children with subglottic inflammation and respiratory distress.

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TRACHEAL RECONSTRUCTION WITH A SYNTHETIC MATERIAL IN A PORCINE MODEL

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The technique of tracheal reconstruction with costal cartilage or a pericardial patch has resulted in successful management of many patients with tracheal stenosis. Yet, it is often difficult, if not impossible, to harvest enough cartilage in a neonate. Pericardial patches are flimsy and can collapse. This pilot study evaluated the use of absorbable plates made of an alloplastic material, Lactosorb, as a substitute for cartilage on a growing porcine trachea. The rectangular plates were fashioned into an inverted U and placed into the airway through an anterior tracheal split. This placement resulted in granulation formation and failure. The plates were then fashioned into an I configuration and placed inside and outside the tracheal lumen. This attempt also resulted in failure, secondary to respiratory distress. Finally, the plates were fashioned into an inverted U and placed outside the trachea. In 67% of the subjects, the distraction was maintained and growth continued for the duration of the study. The stent was completely absorbed, with minimal scar or granulation tissue, in 4 months. In 1 subject, the stent migrated, and 1 subject died of a mucous plug. This study suggests that Lactosorb has no role in intraluminal stenting, but that it may be a viable alternative to a pericardial patch or cartilage in tracheal reconstruction when it is placed outside the airway lumen in a young animal.

KEY WORDS — subglottic stenosis, synthetic material, tracheal reconstruction.

INTRODUCTION

Subglottic stenosis and tracheal stenosis can be congenital or acquired. Generally, acquired cases are a result of endotracheal intubation, but, high tracheostomy, tumor, and trauma are also causes of subglottic stenosis.

The management of subglottic and tracheal stenosis has been controversial. The traditional treatment was tracheostomy, but this treatment option had many well-known complications. These included bleeding, pneumothorax, mucous plugging, delayed speech and language, and a mortality rate of 2% to 24%.[1-6]

Cotton and Seid[7] first described the anterior cricoid split operation in 1980 as an alternative to tracheostomy. The cricoid cartilage and, generally, the first 2 tracheal rings were incised. The airway was stented open with an endotracheal tube for approximately 2 to 3 weeks. The patients usually needed to be sedated or paralyzed during the splinting period, and this requirement can result in complications such as pneumothorax, accidental extubation, and mucous plugging.[8] The reported success rate was approximately 75%.

Alternatively, Prescott[9] developed anterior laryngotracheoplasty, which involved an anterior cricoid split with an inserted cartilage graft. This procedure allowed early extubation without stenting of the airway. The reported success rate was 70% to 80%. This procedure further evolved into the anterior-and-posterior graft, followed by the 4-quadrant graft. All of these techniques required harvesting of cartilage. The usual source was costal cartilage. In neonates, it can be difficult to obtain enough cartilage for stenting, and the success rate is not as good as in older children. Reported complications include donor site wound infections, pneumothorax, and scarring. To eliminate these complications, other materials for grafting have been tried. These included cadaver cartilage, demineralized bovine bone matrix, hydroxyapatite, pericardium, polyethylene, Teflon, and titanium miniplates.[10-14] Adequate results were obtained with most of these materials, but most of these materials were not used to expand growing laryngeal cartilage. Mitskavich et al[11] used titanium plates to expand growing cartilage, but the plates were found to intrude into the airway after the screws loosened with the animal's growth.

Another surgical development involved tracheal resection with end-to-end anastomosis.[15] Accurate approximation of the cut ends of the trachea is a nec-

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From the Department of Otolaryngology–Head and Neck Surgery, University of Minnesota, Minneapolis, Minnesota. Partial funding of the project was provided by Walter Lorenz Surgical. The study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Hennepin County Medical Center.

Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

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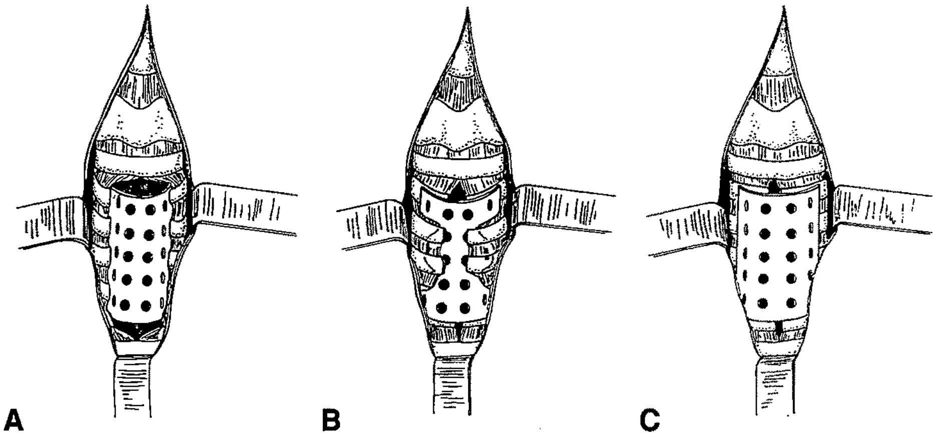

Fig 1. Stent placement. **A)** Completely inside lumen, in inverted U configuration. **B)** Both inside and outside lumen, in I configuration. **C)** Completely outside lumen, in inverted U configuration.

essary step, and the trachea cannot be under tension. A gap of as much as 3 cm can be closed primarily, but as much as 6 cm of the trachea can reportedly be mobilized superiorly with a transthoracic hilar dissection method. Another similar procedure, developed by Goldstraw et al, is a slide tracheoplasty.[16,17] The long segment of the stenosis was divided transversely in its midpoint, a vertical anterior incision was made, and the two ends were slid together, doubling the circumference and shortening the stenotic segment by half. Complications of these techniques have included restenosis, pneumothorax, vocal cord paralysis, anastomosis breakdown, granulation tissue formation, and esophageal perforation.

The resultant restenosis of the airway requires further procedures, such as repeated dilations or intraluminal stents. Presently, intraluminal stents are made of 2 materials: metal wire and silicone. These stents have several potential complications, such as erosion, dislocation, lumen reduction, and the necessity for eventual removal.[18-20]

Thus, it would be ideal to develop an alloplastic material that could stent open the airway without the morbidity of the stents mentioned above. The stent would need to be strong enough to stent open the airway. Lactosorb is a copolymer of poly-L-lactic acid (82%) and polyglycolic acid (18%).[21] It is similar in strength to titanium when it is initially inserted into bone, but it disappears within 9 to 15 months. This is a pilot study that evaluated the tolerance of a growing porcine airway to Lactosorb (Walter Lorenz Surgical) stents 1) inside the trachea, 2) both inside and outside the trachea, and 3) completely outside the trachea.

MATERIALS AND METHODS

Yucatan micropigs were chosen as the experimental animal model because of their many similarities to humans in neck anatomy. They have also been used successfully in previous, similar experimental studies. Micropigs have tracheas similar in size to those of human infants. They mature to their adult size in approximately 5 to 6 months and grow to about 30 to 40 kg in size.

General anesthesia was obtained in 14 young, healthy micropigs with intramuscular injection of xylazine hydrochloride (5 mg/kg) and ketamine hydrochloride (35 mg/kg). An intravenous line was then established, and xylazine hydrochloride (2.5 mg/kg) and ketamine hydrochloride (17.5 mg/kg) were given intravenously every 30 minutes and on an as-needed basis, depending on reflexes. The animal was then given an intramuscular dose of oxytetracycline (10 mg/kg). The animals first underwent laryngoscopy and bronchoscopy with observation for any abnormalities of their airways. The neck was shaved, prepared with Betadine, and draped in a sterile fashion. After careful extension of the neck, the cricoid cartilage was palpated and the skin and subcutaneous tissue were incised vertically to the level of the cricoid cartilage and trachea. The second through sixth tracheal rings were vertically incised. An intraluminal Lactosorb stent was fashioned, in an inverted U or I configuration, from Lactosorb mesh. This stent was then placed completely inside the lumen, partly inside and partly outside the lumen, or completely outside the lumen (Fig 1) and sutured into place with 2-0 Vicryl suture material. The muscles were reapproximated, and a meticulous layered closure was performed.

The piglets were monitored at least twice daily during the first postoperative week. They were then monitored at least once daily. They then underwent bronchoscopic examination at the sixth postoperative week to observe for granulation tissue, bronchial plugging, or signs of aspiration of the Lactosorb material.

The animals that survived were painlessly sacrificed 4 months after the operation. We first performed bronchoscopy to look for tracheal damage. The larynx and trachea were removed, fixed in formalde-

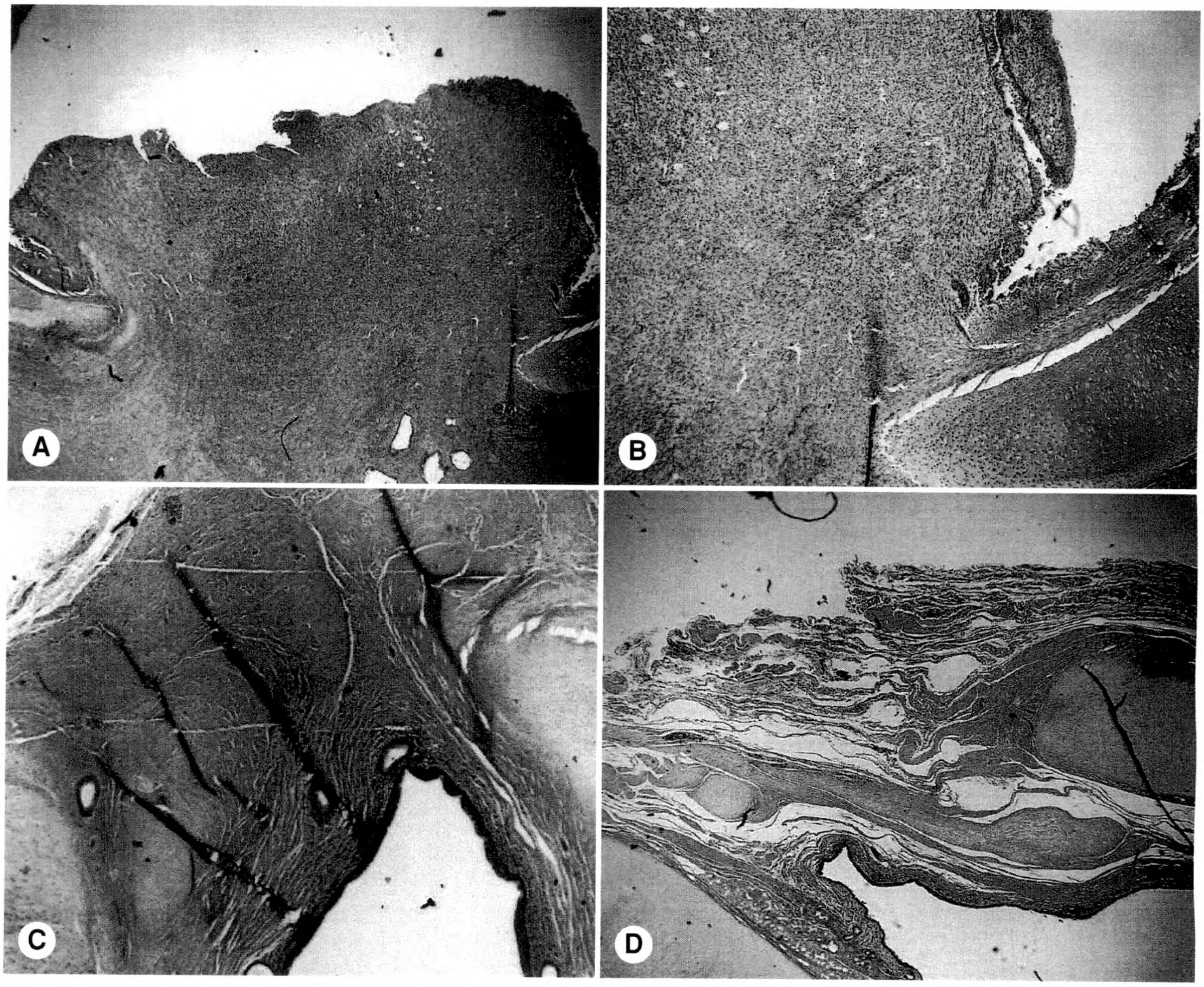

Fig 2. Histologic findings (original ×10). **A,B**) Granulation tissue found at each end of stent when stent was placed completely inside lumen. **C,D**) Collagen formation between cut cartilaginous tracheal rings, with normal respiratory epithelium and no evidence of granulation tissue formation.

hyde, embedded in paraffin for sectioning, and submitted to histologic examination.

RESULTS

The first operations were intraluminal placement of the stent in an inverted U configuration (Fig 1A) in 2 animals. The animals survived approximately 1 week. At autopsy, they were both found to have granulation tissue at each end of the stent (Fig 2A,B).

The stent was then revised to an I configuration, and we placed the horizontal portions of the I outside the lumen and the vertical portion of the I inside the lumen in 6 animals (Fig 1B). Two animals died of viral diarrhea on the fifth postoperative day. The remaining 4 animals died 3 to 6 weeks after the operation, when the stent broke down, migrated distally, and caused lung consolidation and death.

The stent design was changed back to an inverted U, and stents were placed completely outside the lumen in 6 animals (Fig 1C). One animal died of a mucous plug on the second postoperative day. Another animal died of respiratory distress when the stent migrated, 4 weeks after the operation. The 4 remaining animals thrived, grew with no evidence of distress or stridor, and were painlessly sacrificed 4 months after the operation. They underwent bronchoscopy at 6 weeks and 4 months after the operation. During bronchoscopy, there was a well-mucosalized stent region with no evidence of granulation tissue or stenosis. At sacrifice, there was no evidence of residual stent material.

Histologically, there was collagen formation between the cut edges of the trachea. Again, there was no evidence of residual stent material. The lining of the trachea, in the stented area, revealed normal respiratory epithelium (Fig 2C,D). There was no evidence of granulation tissue formation in any of the specimens of the animals that had the stent placed outside the lumen.

DISCUSSION

Tracheal expansion with absorbable plates would ideally allow expansion of the trachea without morbidity to the donor site. The procedure could also be performed on young children, because no cartilage would need to be harvested. Because the plates would be absorbed, there would be a decreased chance of migration. Mitskavich et al[11] showed that titanium plate rigid distraction of the growing, stenotic airway was successful, but eventually, the plates migrated into the airway.

The goal of this pilot study was to evaluate the effects of Lactosorb inside the lumen, partly inside the lumen, and, finally, outside the lumen. The Yucatan micropig was chosen because the size of its airway is similar to that of human infants. The animals were kept alive for 4 months, so we could see whether the stent would actually be reabsorbed and evaluate the effect of the stent on a growing airway. The experiment was not performed on a stenosed airway, as it would have been difficult to evaluate whether an inflammatory response was caused by stent absorption or caused by the procedure that created the stenosis itself.

Our experience strongly suggests that there is no role for Lactosorb in intraluminal stenting, as it causes significant granulation tissue formation. As the stent is absorbed, it also breaks off and migrates distally, causing consolidation in the lungs and the death of the animal.

Endoscopically, Lactosorb plates appear to be tolerated well when placed outside the lumen in a growing airway. In the literature, there are many reports of granulation tissue formation that was caused by air hitting a raw surface when the surface was not mucosalized. This did not appear to happen in our study. The plates seemed to be a good template for new respiratory epithelium formation, without causing granulation tissue. They also seemed to maintain the split, although there were no control animals for comparison. There was no evidence of stridor, with or without activity, and the animals grew, thrived, and developed normally. On bronchoscopic examination, the trachea in the expanded area was larger than the proximal and distal parts of the trachea.

The Lactosorb plates are made of a patented copolymer of poly-L-lactic acid and polyglycolic acid. The copolymer is primarily degraded by hydrolysis into lactic acid and glycolic acid. The Krebs cycle further degrades these products into carbon dioxide and water. Lactosorb, when used in craniofacial surgery, is absorbed in 9 to 12 months. In our study, the plates were completely absorbed in 4 months. There was no evidence of residual plates on macroscopic or microscopic examination. There was only minimal evidence of inflammation on microscopic examination. The rapid absorption of the plates could have been secondary to the profuse blood supply of the peritracheal region.

In summary, the technique of expanding a growing airway with Lactosorb may be a viable alternative to a pericardial patch or cartilage for tracheal expansion in a young animal. Further studies, including expanding a stenosed growing airway, using thicker plates to prolong the time of the expansion, and using control animals, will need to be done before this technique is used in humans. This study indicates that Lactosorb has no role in intraluminal stenting.

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COSMETIC FACIAL SURGERY

The Indian Society of Facial Plastic and Reconstructive Surgery Inaugural Conference and Workshop on Cosmetic Facial Surgery will be held December 7-9, 2001, in Indore, India. For information, please contact Dr Subramaniam Iyyer (subu@md3.vsnl.net.in) or Dr Brajendra Baser, 109 Trade House South Tukoganj Indore MP 452001, India (brajendrab11@hotmail.com, baser@vsnl.com).

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PEDIATRIC BILATERAL VOCAL FOLD IMMOBILITY: THE ROLE OF CARBON DIOXIDE LASER POSTERIOR TRANSVERSE PARTIAL CORDECTOMY

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Vocal fold paralysis in children has been estimated to be the second most common congenital abnormality of the pediatric airway. Most commonly, children present with the vocal folds in the midline or adducted position. Pediatric patients usually have a good voice or cry and a poor airway. In these cases, the surgical goal will be improving the airway, usually by lateralizing a vocal fold, while maintaining a normal voice and intact swallowing. The focus of this article is a modification of a technique for use in adults that was introduced by Dennis and Kashima in 1989. The procedure is intended to separate the vocal ligament and vocalis muscle from the arytenoid cartilage with removal of significant tissue mass with the CO2 laser. The demographics and results of 5 pediatric patients who underwent this procedure are discussed. The preoperative goals of airway enlargement, voice preservation, and normal deglutition were achieved in all 5 patients.

KEY WORDS — carbon dioxide laser, cordectomy, vocal cord paralysis, vocal fold immobility.

INTRODUCTION

Vocal fold paralysis in children has been estimated to be the second most common congenital abnormality of the pediatric airway.[1] This often-quoted statistic may need to be revisited, as the improved survival rate of very low–birth weight premature infants and advances in the techniques used to verify vocal fold paralysis may increase the reporting of this diagnosis. The management of these patients has been challenging and continues to be so. Traditionally, there have been 3 approaches to bilateral vocal fold paralysis in the midline position: tracheotomy, vocal fold lateralization, and reanimation techniques. This report is intended to present an adaptation of the CO2 laser transverse partial cordotomy used in adults by Kashima,[2] for its use in the pediatric patient with bilateral vocal fold immobility. We refer to this procedure as a CO2 laser posterior transverse partial cordectomy, since it enlarges the airway by separating the vocal ligament and vocalis muscle from the arytenoid cartilage and removes a small portion of the posterior part of a vocal fold.

MATERIALS

Five patients (ages 14 months to 13 years; 3 girls and 2 boys) have undergone CO2 laser transverse partial cordectomy. The youngest 3 patients had bilateral vocal fold motion impairment (BVFMI) due to congenital causes, including 1 Arnold-Chiari malformation, and the 2 oldest patients had BVFMI and vocal fold fixation following motor vehicle accidents. Four of the patients had undergone tracheotomy before the procedure. Three had tracheotomy tubes in place at the time of surgery, and 1 had been decannulated at another institution. This patient, although decannulated, had severe dyspnea on exertion, shortness of breath, and limited activity due to airway compromise. The fifth patient, 15 months of age, had been scheduled for a tracheotomy due to respiratory difficulties, and her parents agreed to transverse partial laser cordectomy as an attempt to avoid tracheotomy (Table 1).

The pediatric transverse partial laser cordectomy is performed via suspension microlaryngoscopy using the CO2 laser. The determination of which vocal fold is most suitable for laser treatment is made either during awake flexible laryngoscopy or under spontaneous ventilation anesthesia. If one vocal fold has any degree of motion, the opposite side is selected for surgery. Palpation of the mobility of each arytenoid cartilage is also carried out. The procedure would preferably be performed on the side with the least vocal fold motion and arytenoid mobility. The larynx is sprayed with 2% or 4% lidocaine hydrochloride, depending on the patient's weight. Anesthesia is administered via the tracheotomy tube if one is present, or via a Hunsaker jet ventilation tube or spontaneous ventilation. The appropriately sized laryngoscope is inserted to expose the full endolarynx and

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Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 24-25, 1999.

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TABLE 1. PATIENT DATA

Patient	*Age*	*Sex*	*Cause of Vocal Fold Immobility*	*Tracheotomy at Time of Surgery*	*Decannulated*	*No. of Procedures*	*Complications*	*Procedure Duration (min)*
1	4 y	F	Congenital Arnold-Chiari malformation	Yes	Yes	3	Granuloma	1.42 2.38 3.22
2	5 y	F	Congenital, cause unknown	Yes	Yes	2		1.38 2.40
3	12 y	M	Motor vehicle accident 1 year earlier	Yes	Yes	1		48
4	13 y	M	Motor vehicle accident	No	Not applicable	1		40
5	14 mo	F	Congenital, cause unknown	No	Not applicable	1		30

is suspended from the suspension table. The patient's eyes and face are protected with moistened towels, and all personnel wear protective glasses.

The endoscopic suction is used to retract a vocal fold to improve the egress of the inspired jet ventilation. Using the CO2 laser at 4 W superpulsed continuous power in precise focus, we delineate a posterior triangular wedge consisting of the posterior third of the paralyzed true vocal fold (anterior to the vocal process of the arytenoid cartilage), the false vocal fold, and the ventricle. The dissection is carried out laterally to the level of the thyroid cartilage with vertical extension from the superior level of the false vocal fold to the immediate undersurface of the true vocal fold. This incision frees the vocal ligament and vocalis muscle from the arytenoid cartilage. The posterior extent of the resection is the arytenoid cartilage. The arytenoid cartilage is not exposed, and the vocal process remains intact. This resection creates a triangular defect in the posterior area, enlarging the glottis, while maintaining good approximation of the vocal folds in the anterior commissure (Fig 1). The vocal folds are again misted with lidocaine spray. The patient is monitored in a mist tent for 24 hours and placed on reflux medication for 6 weeks and on an oral antibiotic for 2 weeks.

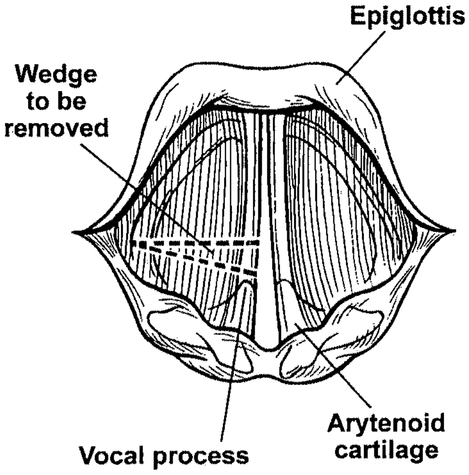

Fig 1. Schematic of surgical approach to CO2 posterior transverse partial cordectomy, shown on left vocal fold.

RESULTS

In this group of 5 patients, a total of 8 procedures were performed. The first patient had 3 procedures, while patients 3 through 5 had only 1 procedure, reflecting the surgeons' learning curve. Perioperative tracheotomy seems not to be necessary, as 2 of these patients did not require it. All of the patients who had tracheotomies before the procedure have been successfully decannulated, with a follow-up of 6 months to 2 years. Our small cohort includes patients with congenital vocal fold paralysis presumed to be neurogenic, as well as patients with acquired bilateral vocal fold fixation due to cervical trauma resulting from a motor vehicle accident. Although the cause of the BVFMI is quite different between these two groups, both tolerated the procedures well and had successful outcomes.

Surgical outcome was assessed in 3 categories: airway, voice, and swallowing. The postoperative airway was rated as good if the patient was decannulated if previously tracheotomized and if there was no dyspnea on exertion or shortness of breath. In tracheotomized patients, the decannulation process was initiated after reexamination of the larynx 6 weeks after surgery. If the airway chink was thought to be adequate, the tracheotomy tube size was decreased and the patient was instructed to keep the tracheotomy tube plugged for all awake hours as tolerated. When the patient was able to keep the tracheotomy tube plugged for 2 weeks, the patient was decannulated in the hospital. The voice was subjectively rated by the parent, and patient when applicable, as normal = 0, mild problem = 1, moderate problem = 2, or severe problem = 3. Swallowing difficulties were assessed as none = 0, mild = 1, moderate = 2, or severe = 3. Unfortunately, formal preoperative and postoperative voice analyses were not carried out. Preoperative and postoperative ratings for each patient are detailed in Table 2. The only complication occurred in 1 patient, who developed a vocal fold granuloma

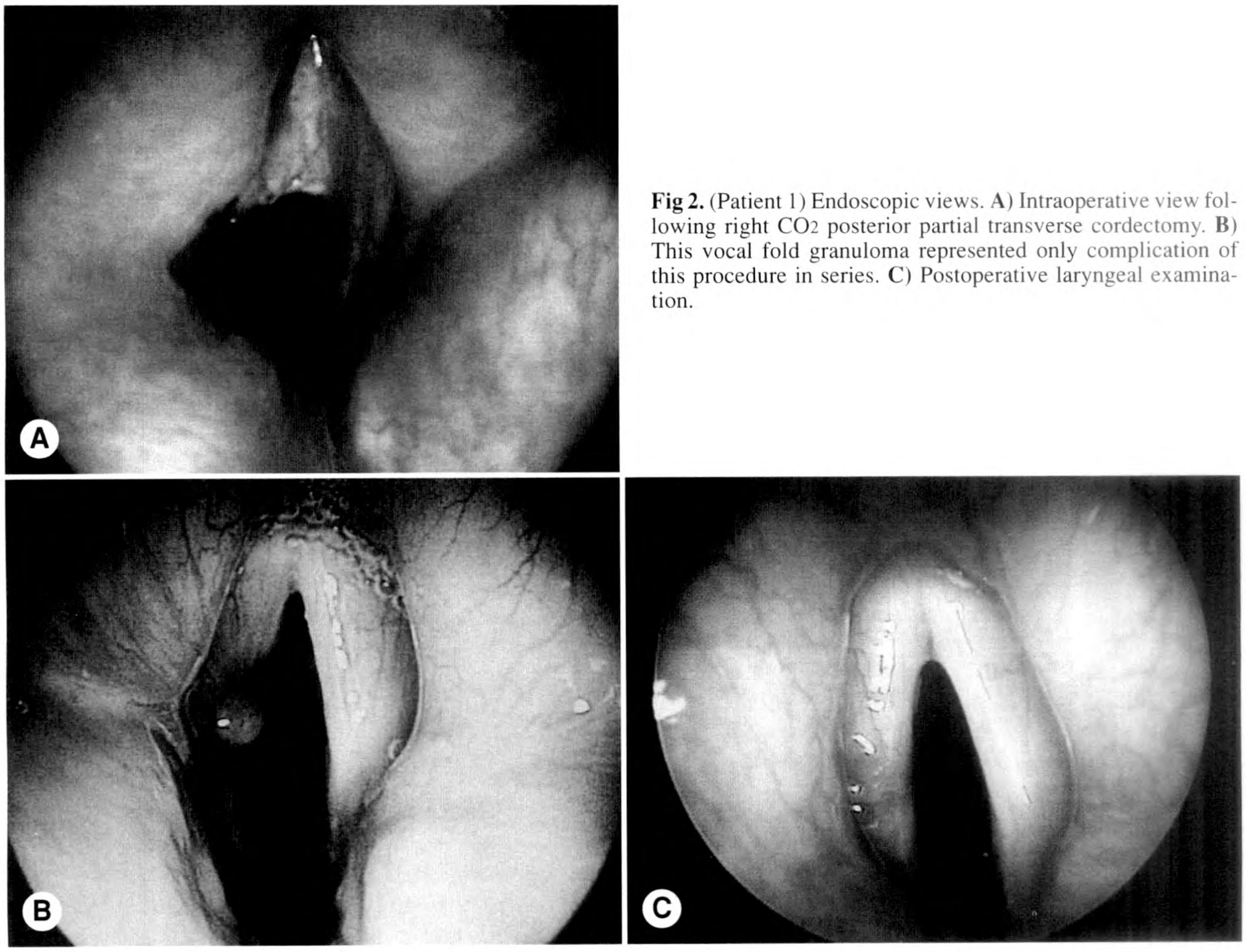

Fig 2. (Patient 1) Endoscopic views. **A)** Intraoperative view following right CO2 posterior partial transverse cordectomy. **B)** This vocal fold granuloma represented only complication of this procedure in series. **C)** Postoperative laryngeal examination.

that was removed successfully with a microlaryngeal scissor dissection (Fig 2B). The parental and patient perception was that the 3 goals of this surgical procedure — improvement of airway (decannulation), satisfactory voice, and no aspiration — were attained in all 5 patients.

DISCUSSION

Vocal fold paralysis in children is inherently quite different from vocal fold paralysis in adults. Most commonly, vocal fold paralysis in adults has a peripheral cause such as surgical trauma or cervical neoplasms causing the paralyzed folds to be in the abducted position.[3] This results in an excellent airway but an unsatisfactory voice. Treatment options are many for these patients, the goal being the medialization or augmentation of one or both vocal folds.[4] In distinction, in the pediatric population, the most common cause of vocal fold paralysis is a central nervous system abnormality such as a meningomyelocele or an Arnold-Chiari malformation.[5] Most commonly, therefore, children present with the vocal folds in the midline or adducted position. Pediatric patients usually have a good voice or cry and a poor airway. In these cases, the surgical goal will be aimed at improving the airway, usually by lateralizing the vocal fold.

Although the term vocal fold paralysis is often used broadly, Kashima[6] has refined the terminology surrounding this condition. He uses the term bilateral vocal fold motion impairment (BVFMI) to more cor-

TABLE 2. SURGICAL OUTCOMES

	Patient 1		*Patient 2*		*Patient 3*		*Patient 4*		*Patient 5*	
	Preop	*Postop*	*Preop*	*Postop*	*Preop*	*Postop*	*Preop*	*Postop*	*Preop*	*Postop*
Airway	Trach	Good	Trach	Good	Trach	Good	DOE, SOB	Good	DOE, SOB	Good
Voice	1	0	1	0	1	1	0	0	2	1
Swallowing	0	0	0	0	0	0	0	0	0	0

Rating system is described in text.
Trach — tracheotomy, DOE — dyspnea on exertion, SOB — shortness of breath.

rectly encompass patients with 1) vocal fold paralysis, 2) cricoarytenoid joint fixation, 3) vocal fold infiltrative lesions, and 4) cicatricial webs. Kashima goes on to state that the 4 forms of BVFMI are indistinguishable on a clinical basis. Regardless of the cause, the resultant condition usually leads to a severely limited airway.

Beyond these issues are the challenges that exist in establishing the correct diagnosis in infants and children. Examination of the larynx when the patient is breathing spontaneously is critical. This may be achieved with direct or flexible laryngoscopy. Direct laryngoscopy under anesthesia may be misleading, even if the patient is not paralyzed, if the plane of anesthesia eliminates spontaneous ventilation or the positioning of the laryngoscope impedes vocal fold movement. Awake flexible laryngoscopy in the infant or child may be too brief or too poorly tolerated to fully assess vocal fold mobility.[7]

After the diagnosis of BVFMI is made, the management in children is also controversial. Several authors have suggested that patients with bilateral vocal fold paralysis can cope well without a tracheotomy or other intervention.[8] This has not been our experience. As neonates become more active and increase their exertion, the airway obstruction usually becomes more limiting, mandating airway intervention. Three management approaches to adult or pediatric patients with bilateral midline vocal fold paralysis are usually considered: long-term tracheotomy, static lateralization, and dynamic reanimation.

For many years, children with bilateral vocal fold paralysis were submitted to tracheotomy. It was thought that although there were risks associated with pediatric tracheotomy, this approach would be the safest, least aggressive intervention. Because the tracheotomy is reversible, spontaneous resolution of the vocal fold dysfunction would result in planned decannulation. Several authors have noted that approximately 50% of children with vocal fold paralysis require a tracheotomy for 3 years or longer.[9] With the introduction of other surgical approaches, tracheotomy is no longer considered a satisfactory long-term treatment. The negative impact of a tracheotomy on the patient and the patient's family or caregivers has made this a least-favored option. Dynamic reanimation continues to be an intriguing possibility, although it has not attained practical application.[10] These procedures, as well as electrical laryngeal stimulators, hold promise; however, currently, these techniques are not practical options.

Static enlargement of the glottis can be achieved externally or endoscopically. External static approaches include a lateral cervical approach, a translaryngeal approach, and laryngofissure.[11-13] The most well known and commonly performed of these extralaryngeal procedures is the Woodman procedure.[14] This procedure is a lateral external approach that avoids violation of the anterior commissure and removes a portion of the arytenoid cartilage. The remaining portion of the arytenoid cartilage is secured laterally to the thyroid cartilage. In spite of the common use of the Woodman procedure and other external procedures in adults, they are performed only occasionally in children. Precise suture placement in children is particularly challenging, and scar tissue formation is of particular concern in the pediatric airway because of the smaller glottic dimensions. Additionally, Bigenzahn and Hoefler[15] have stated that external approaches have produced poorer vocal outcomes than endoscopic techniques, so that these approaches are less desirable.

The endolaryngeal or endoscopic removal of the arytenoid cartilage in adults was popularized by Thornell.[16] The major advantages of this technique were direct visualization, avoidance of the anterior commissure, and the lack of an external incision. The endoscopic exposure and the release of the arytenoid ligaments, however, are challenging and time-consuming. Use of this approach in children is even more problematic, because of limited availability of appropriately sized instrumentation, a small operative field, and subsequent postoperative swelling.[17] Bleeding from the internal laryngeal artery makes the dissection even more difficult. The normal scarring following this type of laryngeal surgery in the small pediatric airway can limit the resultant augmentation of the laryngeal inlet and lead to decannulation failure.

Since the introduction of laser technology into clinical use, the CO_2 laser has played a major role in airway procedures. In the early 1980s, the CO_2 laser was introduced for the endolaryngeal management of bilateral vocal fold paralysis in adults.[18] A variety of different procedures, including laser arytenoidectomy, laser vocal process resection, and laser partial cordectomy, have been used. Although various authors have reported good to excellent results with CO_2 arytenoidectomy, this procedure is difficult.[19-21] The endoscopic exposure of the arytenoid cartilage and vaporization of its ligamentous attachments are often difficult. Therefore, Ossoff et al[22] recommended laser ablation of the arytenoid cartilage, which was thought to be technically easier than excising it. This technique is also very time-consuming and may result in an unpredictable and unquantifiable amount of thermal damage to the surrounding tissue with increased scarring. The scarring may contract the vocal fold medially instead of laterally, limiting the airway. A more recent approach to the endoscopic restoration of an adequate airway is endolaryngeal laser partial cordotomy.[23-25]

Laser cordotomy has been used effectively in adults. In 1989, Dennis and Kashima[26] described CO2 laser endoscopic posterior partial cordotomy. This procedure is intended to separate the vocal ligament and vocalis muscle from the arytenoid cartilage without the removal of significant tissue mass. These authors noted that when the majority of the anterior vocal fold was preserved, the airway could be adequately enlarged with good vocal preservation. They described 6 adult patients in whom this procedure was used to produce reliable enlargement of the laryngeal airway with satisfactory voice. Kashima[2] estimated that in adults, this procedure effectively triples or quadruples the previous cross-sectional area of the glottic lumen. Eckel et al[17] prospectively compared 18 adults undergoing laser subtotal cordectomy to 10 adults undergoing laser arytenoidectomy. The postoperative outcomes were compared between groups. They concluded that both methods were equally effective and reliable, although they believed that laser partial cordectomy was easier and faster.[17] Personal communication with Dr Kashima has resulted in several modifications of his techniques to result in the technique reported in the current review.

Another related method using the CO2 laser is endolaryngeal vocal process resection. First described by Bigenzahn and Hoefler,[15] this method is considered to be a minimally invasive procedure. It involves vaporizing the vocal process of the arytenoid cartilage and the creation of a posterior glottic triangular defect, resulting in glottic airway enlargement. These authors report that bilateral procedures can also be performed in select cases with minimal voice compromise.

Bilateral vocal fold motion impairment is a challenging problem. The difficulties of establishing the diagnosis are compounded by selecting the most appropriate and successful intervention. In this series, all of the children underwent flexible laryngoscopy, as well as direct laryngoscopy with spontaneous ventilation, to establish the diagnosis and determine the most appropriate side for surgery. Since 15% to 60% of children with bilateral vocal fold paralysis will regain some degree of vocal fold function, a definitive surgical procedure should not be considered until the child has been followed for an adequate period of time to observe spontaneous laryngeal recovery.[1,5,8,27] Although the timing in children has not been scientifically addressed, the consensus is that an appropriate interval between diagnosis and surgery would be 6 to 12 months. A longer interval may be warranted in congenital cases. In this series, the youngest patient had been followed 14 months since diagnosis, and the 2 patients with acquired BVFMI had been followed 1 year and 7 years before intervention.

The CO2 laser partial cordotomy has been used successfully in adults for approximately 10 years. Much of the appeal of this technique arises from the inherent characteristics of the CO2 laser, namely, hemostasis, precision, and the ability to carry out the procedure endolaryngeally without extensive "hands-on" manipulation. These characteristics were demonstrated in all 5 of our cases. This technique is minimally traumatic, and there is mild postoperative edema and scarring. Additionally, the CO2 laser transverse partial cordectomy is a practical approach, as it is rapid, well tolerated, and highly successful. In our series, these procedures produced competent, safe airways with good to excellent vocal preservation and no swallowing dysfunction.

There was only 1 complication in this series. One patient developed a vocal granuloma that responded well to sharp excision. An acknowledged concern with this procedure, as in all of the other laryngeal expansion procedures, is the inability to determine in an individual patient the precise amount of tissue to remove in order to attain one's preoperative goals. It can be difficult to predict the necessary balance between airway enlargement and voice preservation in children. The prudent surgeon will therefore undercorrect, bearing in mind that undercorrection may increase the likelihood of additional procedures. Since the morbidity associated with these procedures to date has been small, this cautious approach seems reasonable. Two of our patients required additional procedures, and both families were receptive to repetitive procedures in this setting. Our series of patients, though small in number, illustrates the flexibility of the procedure, with successful outcomes in patients with congenital and acquired vocal cord paralyses, as well as in patients with and without a tracheotomy in place. The 3 preoperative goals — airway, voice, and swallowing — were considered to be met successfully.

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LARYNGOSPASM AND DIAPHRAGMATIC ARREST IN IMMATURE DOGS AFTER LARYNGEAL ACID EXPOSURE: A POSSIBLE MODEL FOR SUDDEN INFANT DEATH SYNDROME

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Laryngopharyngeal reflux has been proposed as a possible cause of sudden infant death syndrome (SIDS). We investigated the efferent laryngeal and diaphragmatic responses to acid exposure on the laryngeal mucosa using a neonatal canine model. Electromyographic (EMG) recordings from the thyroarytenoid muscle and the diaphragm were measured with hooked-wire electrodes. Reproducible laryngospasm responses occurred in all animals after laryngeal exposure to hydrochloric acid at pH 2.0 or less. Laryngospasm occurred in combination with tachypnea and increased diaphragmatic activity in most of the animals. Laryngospasm was associated with prolonged apnea and total cessation of diaphragmatic EMG activity in 1 animal, and in another, initial tachypnea was followed by erratic diaphragmatic activity and brief apnea. Laryngeal acid exposure (below pH 2.0) causes laryngospasm and may result in paradoxical apneic events in neonatal dogs. Acid-induced, laryngospasm-associated apnea may represent a potential cause of SIDS, and the immature dog appears to be an excellent model for further investigations.

KEY WORDS — apnea, crib death, dog, electromyography, gastroesophageal reflux, infant death, laryngeal reflexes, laryngopharyngeal reflux, laryngospasm, larynx, reflux, sudden infant death syndrome.

INTRODUCTION

Sudden infant death syndrome (SIDS) is defined as the sudden death of an infant less than 1 year of age that remains unexplained after a thorough investigation including postmortem examination of the death scene and review of the clinical history.[1] It remains the leading cause of death among infants from 28 days to 1 year of age.[2] Some of the previously identified risk factors include low birth weight, prematurity, low socioeconomic status, bottle feeding, sleeping position, and male gender.[1-3] The cause of SIDS remains unknown. Proposed causes of this entity include immunodeficiency, allergy, endocrinopathy, and respiratory compromise. Stowens[4] has observed increased lung weight, pulmonary edema, and alveolar changes in infants with SIDS.

The larynx has been implicated as a possible site of airway obstruction. Laryngeal reflexes include the glottic closure reflex, laryngospasm (LS), and the central apnea reflex. Within central reflex arcs, the larynx functions both as a sensory organ with afferent projections to the brain stem via the superior laryngeal nerve (SLN) and as an end organ producing airway compromise via glottal closure.[5] The afferent supply of these reflexes is carried by the SLN. The efferent limb of the glottic closure reflex and LS is through the recurrent laryngeal nerve (RLN), primarily to the thyroarytenoid (TA) muscle.[6] Reflex glottal closure results from swallowing, as well as mechanical, chemical, and thermal stimulation. This reflex serves to protect the airway from aspiration.

Laryngospasm represents an exaggerated glottic closure response resulting from the efferent responses of supraglottic laryngeal chemoreceptors to noxious stimuli. These stimuli produce excitatory discharges of the RLN resulting in sudden, forceful, prolonged apposition of the vocal folds.[6] Sasaki[7] identified central excitatory and inhibitory influences on the LS reflex. In the dog, pups between the ages of 50 and 75 days appear to be at particular risk of LS as a result of neural immaturity and a transient reduction in central inhibition.[7] Several animal studies have shown acid to be a potent stimulator of the LS response.[8-10] Loughlin and Koufman[11] demonstrated in an adult dog model that experimental laryngopharyngeal reflux (LPR) is a potent precipitator of the LS response.

Central reflex apnea results from central inhibition of the respiratory centers in the brain stem via stimu-

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Presented at the meeting of the American Laryngological Association, Orlando, Florida, May 13-14, 2000.

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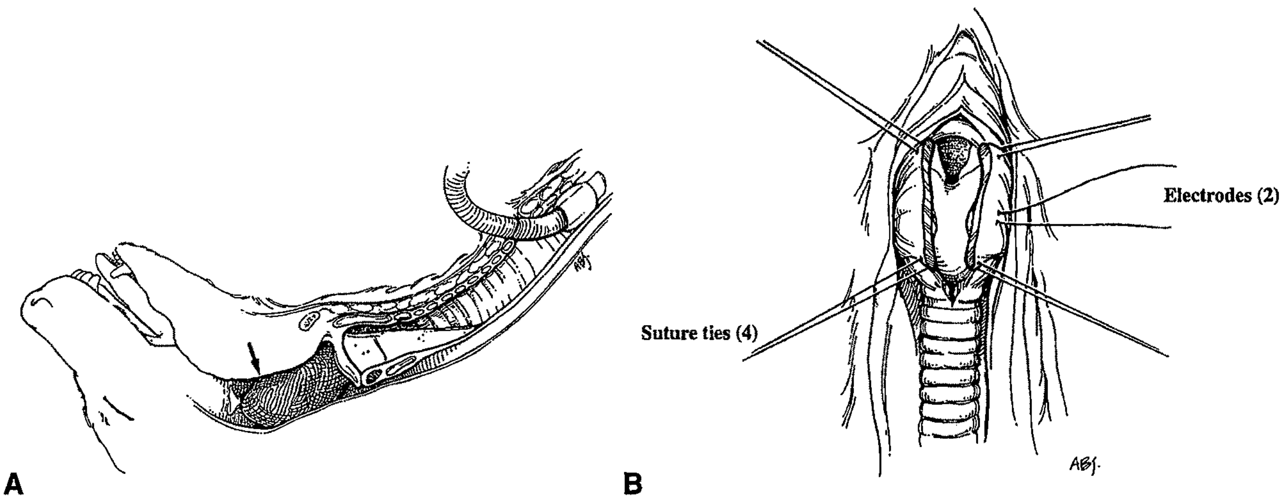

Fig 1. Canine model of laryngospasm (LS). **A)** Omentum (arrow) was placed into hypopharynx to create basin effect in larynx and prevent leakage of test solutions into hypopharynx. **B)** View from above of larynx with hooked-wire electromyographic (EMG) electrodes placed in left thyroarytenoid (TA) muscle.

lation of the SLN. As with LS, the duration of the reflex apnea persists beyond the duration of the stimulus. Lanier et al[10] identified an increased apnea response in the face of hypoxia, decreased pH, and laryngeal exposure to hypotonic saline solution. The central apnea reflex is opposed by the brain stem respiratory drive. In neonatal animals, SLN stimulation outweighs the central respiratory drive; thus, central reflex apnea is often fatal. After sufficient maturation of the central nervous system, the brain stem response to hypoxia and hypercarbia prevents fatal apnea. Lanier et al further observed that fatal apnea secondary to laryngeal acid exposure in neonatal piglets was confined to animals less than 3 weeks of age.

Wetmore[9] observed LS and central apnea in neonatal rabbits exposed to laryngeal acid. On the basis of previous studies, the canine model appears to be more similar to human neuroanatomy than other animal models.[6,12] Loughlin et al[8] demonstrated consistent and highly reproducible LS responses to laryngeal acid exposure in *adult* dogs; however, in none of these mature animals was a central apnea response observed. The purpose of this study was to investigate the effects of experimental reflux on LS and central apnea responses in an *immature or neonatal* canine model.

MATERIALS AND METHODS

Ten neonatal mongrel dogs 19 to 26 days of age were used for these studies, which were performed in accordance with our institution's Animal Care and Use Committee. After induction of general anesthesia with halothane, each animal was orotracheally intubated. Peripheral venous and arterial access was obtained. Continuous pulse oximetry and arterial pressure were monitored throughout the procedure via a Uniflo model 43-MK239 (Baxter Healthcare, Irvine, California) pressure transducer. The core temperature of each study animal was stabilized and maintained at 38°C with a customized warming blanket. Intermittent arterial blood gases were sampled to maintain physiologic oxygen and carbon dioxide levels.

A midline vertical cervical incision was made, and a tracheotomy was performed approximately 1.5 cm below the cricoid cartilage. The ventilatory circuit was then transferred to the tracheotomy, and the endotracheal tube was removed. A midline laryngofissure was performed with a No. 15 scalpel blade, with care taken to avoid injury to the vocal folds. Hooked-wire platinum electromyographic (EMG) electrodes were placed in the left TA muscle by passing the electrodes through the thyroid cartilage, into the belly of the muscle. This manner of insertion preserved the mucosal integrity of the endolarynx (Fig 1A).

A high midline laparotomy was performed. Omentum was harvested and placed in the hypopharynx. This created a basin effect in the larynx within which to place test solutions while preventing leakage into the pharynx (Fig 1B). Hook-wire platinum EMG electrodes were placed in the left diaphragmatic crus. The EMG signals were digitized at 2,000 samples per second and recorded with a Windaq Data Acquisitions System program (Dataq Instruments, Inc, Akron, Ohio). The laparotomy was closed to prevent desiccation of the abdominal contents and minimize insensible fluid losses.

The level of anesthesia was titrated with the concentration of inspired volatile gas to a plane at which phasic respiratory EMG activity was observed in the TA muscle and diaphragm. With deeper planes of

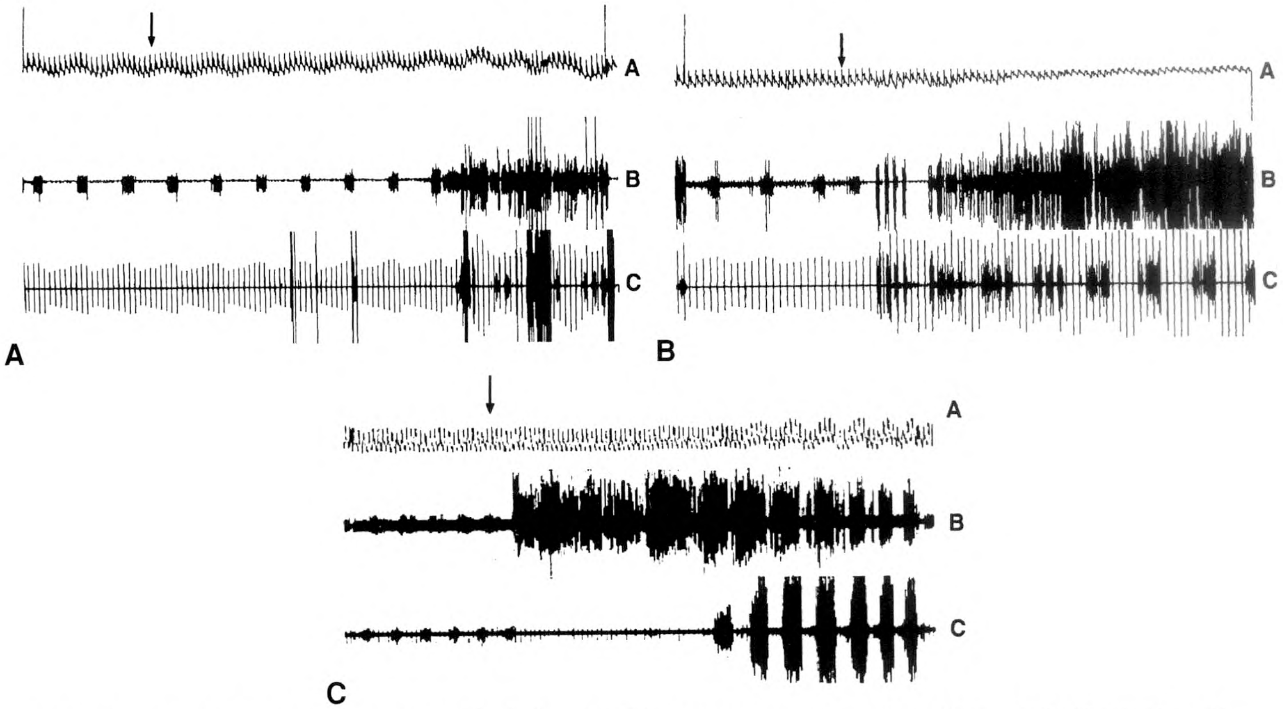

Fig 2. Polygraph tracings of responses to acid solution. A — blood pressure recording; B — TA muscle EMG recording; C — diaphragm EMG recording; arrow — point at which test substance was instilled. **A)** LS response with test substance at pH 2.0. TA muscle showed dramatic increase in firing within 20 seconds of instillation of acid (LS response). At same time, clinical LS was observed. In this animal, diaphragmatic EMG activity increased during LS response. **B)** LS response with test substance at pH 1.0. TA muscle showed dramatic increase in firing within 5 seconds of instillation of acid. In addition, there was increase in diaphragmatic EMG firing during LS. LS response was similar, but more intense than that shown in A (pH 2.0), and latency was shorter. **C)** LS response and apnea with test substance at pH 1.0. TA muscle showed dramatic increase in firing within 3 seconds of instillation of acid. In this animal, LS response was accompanied by complete arrest of diaphragmatic activity (central reflex apnea response). This apnea response lasted more than 15 seconds.

anesthesia, first the diaphragmatic activity was lost, then the TA muscle activity. With shallower planes of anesthesia, coughing and swallowing were noted. The concentration of halothane required to reach the desired level of anesthesia varied with each animal, yet remained within a range of 0.25% to 1.00%.

Once the desired plane of anesthesia was obtained, test solutions were instilled in the larynx. Approximately 10 mL was used to fill the larynx and cover all of the endolaryngeal mucosa. Laryngeal and diaphragmatic EMG signals were recorded during instillation of each test solution. Each solution was removed via suctioning after 2 minutes, and the larynx was irrigated 3 times with phosphate-buffered saline solution (pH 7.4). Baseline EMG activity returned after rinsing in all cases. The solutions tested were acid solutions of pH 4.0, 3.0, 2.0, 1.5, and 1.0. This limited number of test solutions was based on previous experiments that observed LS at pH 2.5 or less.

At the completion of the experimental protocol, each dog was euthanized by lethal injection of potassium chloride.

Test solutions were prepared on the morning of each experiment to ensure an accurate pH. Solutions were kept in a heating bath at 38°C. All solutions were at physiologic temperature before instillation into the larynx. The acid solutions were made by titrating 0.9% sodium chloride to pH 4.0, 3.0, 2.0, 1.5, and 1.0 with 1N hydrochloric acid. A Fisher Accumeter model 610-A pH meter (Denver Instruments Company, Denver, Colorado) was used.

RESULTS

Instillation of phosphate-buffered saline and neutral pH solutions had no effect on baseline recordings of TA and diaphragmatic EMG activity, nor were there any LS responses to hypertonic or hypotonic saline solutions. Laryngospasm responses were elicited in all animals at pH threshold values of 2.5 or lower. The pH threshold for LS was 2.5 in 1 animal, 2.0 in 7 animals, and 1.5 in 2 animals. A rapid increase in EMG firing that culminates with tonic contraction of the TA muscle characterizes this response. In every case, tonic TA activity on EMG recording correlated with observed LS. The LS response was highly reproducible; repeated application of subthreshold acid solutions produced the same LS response in every animal.

The latency between the application of a subthreshold acid solution and LS reflected the pH of the test solution. Latency periods varied directly with the pH — the lower the pH, the shorter the latency — and that relationship was seen in every animal. Figure 2A shows LS occurring in response to acid solution at pH 2.0 with a latency of 20 seconds, and Fig 2B shows LS occurring in response to acid solution at pH 1.0 with a latency of only 5 seconds. Also observed in the tracings shown in Fig 2A,B is increased diaphragmatic EMG activity during LS.

In 8 of the 10 animals, LS was associated with an increased heart rate and an increased diaphragmatic firing rate. In 1 animal, as the pH of the test solution was lowered, the diaphragmatic activity changed. With LS at a pH of 2.5, this animal showed increased diaphragmatic activity. At pH 2.0, the EMG recording of the diaphragm showed irregular activity during LS. However, in this same animal at pH 1.0, complete apnea (arrested diaphragmatic activity on the EMG recording) was associated with LS (Fig 2C). The apnea persisted approximately 15 seconds, whereas LS continued until the larynx was rinsed with phosphate-buffered saline solution. In another animal, application of an acid solution at pH 1.0 was associated with tachypnea followed by an erratic diaphragmatic firing rate and then a brief (5-second) apnea.

DISCUSSION

Our data demonstrated that acid-sensitive chemoreceptors initiated LS at a pH of approximately 2.0; this finding was highly reproducible and consistent with previous reports in the adult canine model. The latency period between acid exposure and LS varied with the strength of stimulation. At maximal stimulation (pH 1.0), this latency period was observed to be as short as 3 seconds (Fig 2C). Notably, in humans, the acidity of the gastric contents is often lower than pH 1.0; pH measurements as low as pH 0.3 are commonly observed clinically during reflux testing, ie, pH monitoring.

In keeping with findings in other species,[9] we have shown that central reflex apnea can occur in response to SLN stimulation via laryngeal chemoreceptors in the immature canine larynx. This response was observed in 2 of our animals. The absence of observed reflex apnea in 8 of the 10 animals might have been due to the maturational stage or other factors. Sasaki[7] indicated that canine pups (50 to 75 days postnatally) exhibited transient reductions in central inhibition. Furthermore, maturation of the laryngeal neuroanatomy as evidenced by RLN myelination and conduction occurs at 30 to 45 days.[6] The animals in our study ranged in age from 19 to 26 days, and it is possible that the central apnea reflex may be more common later in the neonatal period. Indeed, the best example of central reflex apnea in our study came from the oldest animal.

Shatz et al[13] reported observation of basement membrane thickening in the larynges of infants with SIDS. This study suggested the existence of a reliable histologic marker for SIDS and that SIDS might be related to an underlying inflammatory condition. It is interesting to note that the peak (median) age of SIDS occurs at about 8 months. Gastroesophageal reflux, as shown by 24-hour pH monitoring, also peaks at that age.[14] If the peak, in terms of total acid exposure time, occurs at the same time as the greatest period of LS and apnea susceptibility, then SIDS might be explained by a synergistic and potentially deadly simultaneous occurrence of 2 different developmental factors.

Presumably, SIDS occurs during a transitional period in the regulation of certain higher brain stem regulatory functions as proposed by Sasaki et al.[6] Wetmore's[9] report of acid-induced LS and apnea in a neonatal rabbit offered the first animal model for acid-induced LS and SIDS; we offer a second model.

In neonatal animals, central reflex apnea has been shown to be fatal. The immature respiratory centers cannot overcome the SLN-mediated reflex apnea. In this model, mechanical ventilation prevented hypercarbia, hypoxia, and death. As a result, the relationship between reflex apnea and the hypoxia or hypercarbia respiratory drive cannot be determined from this set of experiments. Further, because of the presence of a tracheotomy and omentum in the hypopharynx, gasping and swallowing responses to noxious stimuli were difficult to quantify. Future experiments will address these factors.

Contemporary theories of the cause of SIDS focus on the aerodigestive tract and the larynx in particular. The concept of a prolonged mixed apnea (LS and central apnea) as a result of laryngeal acid exposure, such as in LPR, is a possible explanation for SIDS. Laryngopharyngeal reflux in the human neonate, as shown by Vandenplas and Sacre-Smits,[14] is common, in contradistinction to the relative rarity of SIDS. Unlike the neonatal rabbit model, the canine model similarly demonstrates the central apnea reflex associated with LS to be relatively infrequent. Further studies utilizing the immature canine model are needed, specifically, investigations of the developing neuroanatomy and laryngeal reflex pathways.

CONCLUSIONS

1. Acid-sensitive chemoreceptors present in the immature canine larynx reproducibly initiate LS at a

pH of 2.0 or less.

2. The immature dog may represent a viable model for investigating acid-induced LS and central reflex apnea.
3. Central reflex apnea as a result of laryngeal acid exposure, as in LPR, may be a cause of SIDS.

ACKNOWLEDGMENTS — The authors gratefully acknowledge the contributions of Chris Loughlin, MD, John Little, MD, and Sera Jacob, MD, without which this project would not have been possible.

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Ann Otol Rhinol Laryngol 110:2001

THYROGLOSSAL DUCT CARCINOMA: A LARGE CASE SERIES

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Thyroglossal duct cysts (TDCs) are common congenital abnormalities of thyroid development. Carcinoma occurs rarely in patients with TDCs. In a large case series drawn from medical records at a health maintenance organization from 1971 through 1995, 14 cases of carcinoma in a TDC were found. This represents the largest known reported case series to date. Demographics, diagnosis, and treatment methods are reviewed. We recommend the Sistrunk operation and evaluation of the thyroid gland and neck for potential abnormality that would necessitate thyroidectomy and lymph node dissection. Long-term follow-up incorporating clinical examination is mandatory.

KEY WORDS — head and neck neoplasm, papillary carcinoma, thyroglossal cyst, thyroid neoplasm.

INTRODUCTION

Thyroglossal duct cysts (TDCs) are common congenital abnormalities of thyroid development.[1] Because the thyroglossal tract is related closely to the thyroid gland, thyroid tissue may be found in the cyst or cyst wall.[2] However, development of carcinoma in a TDC is rare: only slightly more than 150 cases have been reported in the biomedical literature in English.[3,4] As with management of malignant thyroid neoplasms in general, controversy also exists regarding management of neoplasms in a TDC. We report an additional 14 cases of carcinoma in a TDC, the largest reported series to date known to us. We note various treatment plans and outcomes of these cases and recommend a preferred treatment plan.

MATERIALS AND METHODS

The International Classification of Diseases[5] codes were obtained for patients discharged in the Northern California Region of the Kaiser Permanente Medical Care Program from 1971 through 1995; 1,075 patients with a diagnosis of TDC were identified. Medical records of patients with simultaneous diagnosis codes of TDC and malignant thyroid neoplasm were reviewed. Thirty patients who met these criteria were identified. Of these patients, 14 had a neoplasm in a TDC.

RESULTS

Tables 1 and 2 expand on the preoperative, operative, and postoperative characteristics of the series of patients. Of the 14 patients, 8 were women and 6 were men. The mean age (± SD) was 39.3 ± 15.0 years (range, 22 to 68 years). In 5 patients, the initial complaint was an enlarging mass in the neck. In 4 patients, an asymptomatic mass in the neck was discovered during routine examination. Three patients noticed a change in a long-standing neck mass or goiter. Two came to the office for evaluation of pain in the neck. The neck masses were present for as long as 15 years before patients sought evaluation. None of the 14 patients had any risk factors for thyroid carcinoma. At examination, 2 patients had a mass in the suprahyoid area, and 12 had a mass located between the hyoid bone and the thyroid cartilage. Most of the masses were firm (n = 10), whereas others were cystic (n = 2) or nodular (n = 1).

Various methods were used for preoperative assessment. Ten patients had nuclear scanning of the thyroid bed. Three (patients 1, 2, and 12) had an area of relatively low uptake near the TDC, and 3 (patients 4, 9, and 11) had an area of increased uptake near the TDC. The others had normal thyroid scan findings near the TDC, and all but 1 patient (patient 5) had scans showing a normal thyroid gland. Five patients underwent fine-needle aspiration (FNA): 2 aspirates showed dark, bloody fluid, and 3 produced results positive for papillary carcinoma. All patients had normal results on thyroid function studies. Various other studies, including ultrasound, computed tomography, and magnetic resonance imaging, were used in smaller numbers.

All patients underwent the Sistrunk procedure except patients 1, 7, and 11, who had only the neck mass excised. Patient 11 had the Sistrunk procedure completed later.

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TABLE 1. PREOPERATIVE CHARACTERISTICS OF PATIENTS IN LARGE CASE SERIES OF THYROGLOSSAL DUCT CYST CARCINOMA

Patient	*Age (y)*	*Sex*	*Risk Factors*	*Symptoms*	*Tumor Location*	*Mass Size*	*Consistency*	*Preoperative Studies*	*Time Before Diagnosis*	*Time Before Surgery*
1	26	F	None	Enlarging mass	Midline upper thyroid	1 cm	Firm, rubbery	NUC: cold nodule near isthmus	18 mo	1 mo
2	36	F	None	Enlarging mass	Left cricoid area	1 × 0.5 cm	Firm	TFT: WNL; ultrasound: WNL; NUC: slightly cool area, left upper pole	1 mo	0.5 mo
3	22	F	None	None	Midline	3 × 1.5 × 1 cm	Unspecified	FNA: amber fluid	12 mo	3 mo
4	27	M	None	Noted incidentally	Over larynx	1.5 × 1.3 × 1 cm	Firm, rubbery	FNA: papillary CA; NUC: thyroid WNL, some uptake in cyst; MRI: bilateral cervical nodes	0 mo	1.5 mo
5	49	F	None	Noted incidentally	Hyoid level slightly to right	2.4 × 1.5 × 1.1 cm	Firm	FNA: papillary CA; NUC: slight uptake in right lower thyroid, none in cyst	0 mo	1.5 mo
6	48	M	None	Goiter for 15 y with change	Right upper thyroid	4 × 2 × 1.5 cm	Nodular	FNA: papillary CA; MRI: cyst and neck mass	15 y (goiter)	1.5 mo
7	27	M	None	Enlarging mass	Right upper thyroid	2.5 × 3 × 2.5 cm	Cystic	CT: mass, right inferior thyroid	2 mo	4 mo
8	56	M	None	None	Left upper thyroid	4.2 × 4 × 2.6 cm	Firm	TFT: WNL; NUC: cyst only	4 mo	1 mo
9	59	M	None	Long history of mass, pain	Suprahyoid	2 × 2.5 × 2 cm	Cystic	NUC: thyroid WNL, slight uptake near cyst	60 mo	1 mo
10	26	F	None	Goiter for 14 y with change	Midline, thyroid cartilage	3.6 × 3 × 2.1 cm	Firm	FNA: bloody fluid; TFT: WNL; NUC: WNL	14 y (goiter)	2 mo
11	28	F	None	Pain	Thyrohyoid membrane	2.3 × 1.7 × 1.5 cm	Firm	NUC: thyroid WNL, slight uptake near cyst; MRI: TDC only	12-24 mo	4 mo
12	68	M	None	Pain	Thyrohyoid membrane	1.5 × 4 × 1 cm	Firm	NUC: thyroid WNL, cold nodule near TDC	6 mo	4 mo
13	30	F	None	None	Suprahyoid	3.2 × 2.2 × 2 cm	Firm	None	0 mo	1 mo
14	48	F	None	Enlarging mass	Thyrohyoid membrane	2 × 2 cm	Firm	NUC: thyroid WNL, no uptake in cyst	1 mo	1 mo

NUC — nuclear thyroid scanning; TFT — thyroid function test; WNL — within normal limits; FNA — fine-needle aspiration; CA — carcinoma; MRI — magnetic resonance imaging; CT — computed tomography; TDC — thyroglossal duct cyst.

In 4 patients (patients 1, 2, 3, and 14), treatment was limited to removal of the TDC. Although 1 patient was unavailable for follow-up after 1 month, none had any treatment complications. No recurrent neck masses were noted during follow-up, and no further surgery was required for any of these patients.

Patient 9 underwent the Sistrunk procedure initially. After close follow-up for nearly 4.5 years, a neck mass developed that was found on excision to be papillary carcinoma of the thyroid gland. The patient underwent total thyroidectomy at the same time, and analysis of the removed thyroid tissue showed no carcinoma. He did well and had no complications or evidence of recurrence after 208 months.

Six patients (patients 7, 8, 10, 11, 12, and 13) underwent elective thyroidectomy after papillary carcinoma was found in the TDC. Four of these patients had no carcinoma in the thyroid gland. One patient

TABLE 2. OPERATIVE AND POSTOPERATIVE CHARACTERISTICS OF PATIENTS IN LARGE CASE SERIES OF THYROGLOSSAL DUCT CYST CARCINOMAS

Patient	*Primary Procedure*	*Interval to Second Procedure*	*Secondary Procedure*	*Tumor Pathology*	*Follow-up Treatment*	*Suppression*	*Follow-up*	*Problems or Complications*
1	Removal of TDC, not hyoid, paratracheal LN	NA	None	Papillary CA replacing TDC, 2 × 1 × 0.4-cm focus of CA in LN	None	No	25 mo	None
2	Sistrunk	NA	None	Tumor replacing TDC	None	Yes	60 mo	None
3	Sistrunk	NA	None	Small focus of CA (0.8 cm) in TDC	None	Yes	60 mo	None
4	Sistrunk, total thyroidectomy, bilateral functional neck dissection	NA	None	Focus (0.7 × 0.6 × 0.5 cm) in TDC, small focus in right thyroid lobe, 3 positive nodes in right side of neck	^{131}I	Yes	14 mo	None
5	Sistrunk, total thyroidectomy	NA	None	Small focus in TDC, 0.25-cm focus in left lobe of thyroid	^{131}I	Yes	16 mo	None
6	Sistrunk, total thyroidectomy, excision of mass in right side of neck	9.5 mo	Bilateral neck exploration, excision of mediastinal mass	Foci in both lobes of thyroid, isthmus, mediastinal mass	^{131}I	Yes	23 mo	Bilateral vocal cord paralysis
7	Excision of cyst	9 mo	Right thyroid lobectomy, partial left lobectomy	Focus of CA in TDC, bilateral thyroid adenomas, no CA in thyroid	^{131}I	Yes	1 mo	None
8	Sistrunk	6.5 mo	Total thyroidectomy	Focus of CA in TDC, no CA in thyroid	^{131}I	Yes	35 mo	None
9	Sistrunk	55 mo	Total thyroidectomy, removal of left LN	5 × 5-cm LN, left side of neck, no CA in thyroid	^{131}I	Yes	208 mo	None
10	Sistrunk, excision of delphian node	0.5 mo	Total thyroidectomy, cervical LN	CA in delphian node, no CA in thyroid, CA in paratracheal LN	^{131}I	Yes	69 mo	Lung metastases
11	Excision of mass	0.5 mo	Completion of Sistrunk, total thyroidectomy	0.2-cm CA in TDC, no CA in thyroid	^{131}I	Yes	55 mo	None
12	Sistrunk	1 mo	Total thyroidectomy	Focus of CA in right thyroid lobe	^{131}I	Yes	50 mo	None
13	Sistrunk	2 mo	Total thyroidectomy	Focus of CA in left upper pole	^{131}I	Yes	150 mo	Hypocalcemia
14	Sistrunk	NA	None	CA replacing TDC	None	No	1 mo	None

TDC — thyroglossal duct cyst; LN — lymph node; NA — not applicable; CA — carcinoma; Sistrunk — Sistrunk procedure; ^{131}I — iodine 131.

(patient 10) also had a paratracheal lymph node that contained papillary carcinoma excised during thyroidectomy. Only 2 of these 6 patients (patients 12 and 13) had occult microscopic foci of carcinoma in the thyroid gland. Two of these patients had complications. Patient 12 had persistent hypocalcemia requiring oral calcium supplementation. In patient 10, lung metastases appeared 5 years after treatment. No other patient had any evidence of recurrent disease.

Three patients underwent more aggressive initial therapy. Patient 4 had preoperative evidence of carcinoma in the TDC and cervical adenopathy bilaterally. He underwent the Sistrunk procedure, total thyroidectomy, and bilateral functional neck dissection. This patient had 3 lymph nodes in the right side of the neck that proved positive for carcinoma and a small focus of carcinoma in the right lobe of the thyroid gland. At the last follow-up visit, he had no evidence of recurrent disease.

In patient 5, FNA showed papillary carcinoma in the TDC, and preoperative thyroid scintigraphy showed an abnormality in the thyroid gland. She underwent the Sistrunk procedure and total thyroidectomy. The pathology report showed a focus of carcinoma in the thyroid gland and in the TDC. The patient was free of disease at 16 months.

Patient 6 also underwent preoperative FNA that showed papillary carcinoma in the TDC. Magnetic resonance imaging showed an enlarged lymph node in the right side of the neck. The patient underwent the Sistrunk procedure, total thyroidectomy, and excision of the mass from the right side of the neck. The pathology report showed carcinoma in the TDC, in both lobes of the thyroid gland, and in the lymph node. After 9.5 months, a mediastinal mass developed. The patient then underwent bilateral neck explorations that confirmed carcinoma in the mediastinal mass only. During treatment, bilateral vocal cord paralysis developed, which required tracheotomy to secure the airway. At follow-up nearly 2 years after treatment, the patient had no evidence of recurrence.

All patients who had thyroidectomy underwent postoperative thyroid ablation with iodine 131 treatment. Twelve of the 14 patients also underwent long-term thyroid suppression treatment.

DISCUSSION

During fetal development, a diverticulum that is destined to become the thyroid gland descends from the tongue base to its eventual position anterior to the trachea.[2] A remnant called the thyroglossal tract is left behind and usually disappears by the seventh fetal week, but may persist.[3] This remnant may also become entrapped within the hyoid bone during development of this bone.[2] Dilatation of a persistent thyroglossal tract may be responsible for formation of a TDC.

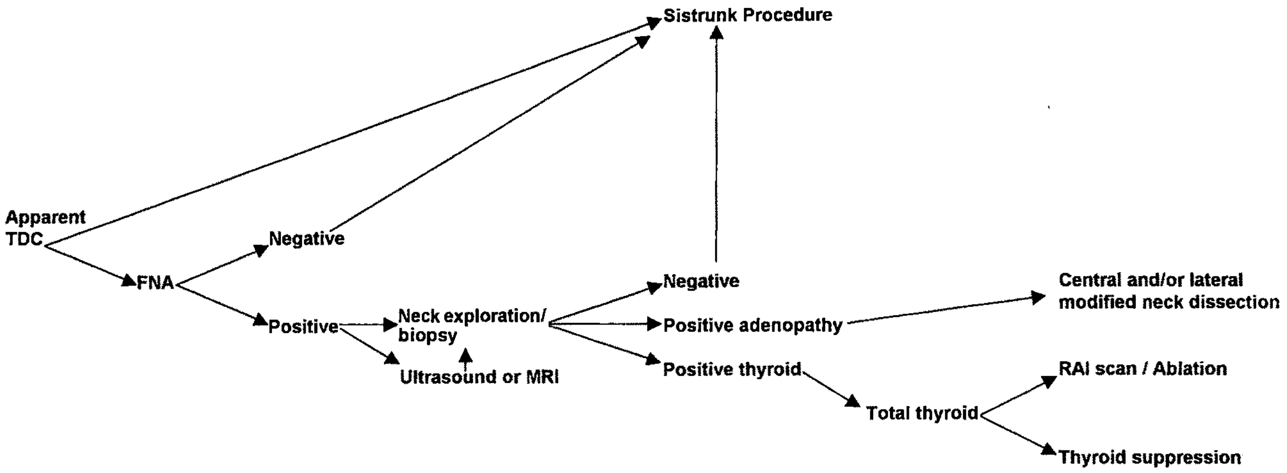
Thyroglossal duct cysts are commonly occurring cervical cysts that occur frequently in young patients and equally in both sexes.[2] They may be asymptomatic unless they become infected. The patients in our group compare with those in other series with respect to age (mean, 39.3 years),[4,6] sex distribution (8 women, 6 men), and severity of symptoms (minimal).[2]

The presence of thyroid tissue in a TDC can be understood on the basis of the embryology of the thyroid gland. Its frequency has been estimated at 1.5% to 45%.[2] Carcinoma rarely occurs in TDCs; about 150 cases have been reported.[1,7] The incidence of carcinoma in our current study is 1.3%. Most TDC carcinomas are papillary[4]; a few patients have follicular, squamous cell,[6] or other types of carcinoma.[4] In our series, all 14 patients had papillary carcinoma.

Preoperative diagnosis of patients with carcinoma in a TDC is unusual. The mass of a TDC carcinoma is often asymptomatic and has often been present for many months or longer when found. Rapid growth may provoke suspicion, but such growth is an unreliable indicator of carcinoma.[3] Carcinoma may be indistinguishable from a benign TDC in terms of location, size, or consistency. In younger patients, nuclear scanning may confirm other functioning thyroid tissue besides that in the TDC. In this series, preoperative thyroid scanning did not seem to be of value in diagnosing carcinoma in the TDC or the thyroid gland. Fine-needle aspiration may be valuable for preoperative planning or counseling if the result is positive for carcinoma. Patients are usually euthyroid. Therefore, carcinoma in a TDC is often diagnosed only after surgery.

Management of carcinoma in a TDC remains controversial. Should the thyroid gland be removed? What is the role of postoperative hormonal suppression or ablation with radioactive iodine? Because of the embryological development of the TDC, the Sistrunk procedure is preferred for treatment. Originally described in 1928 as including resection of the cyst and tract extending into the foramen cecum and including the central portion of the hyoid bone, this operation has become standard practice.[8] In our series, the 4 patients who underwent excision of the TDC only had no recurrence or evidence of disease at follow-up visits or on nuclear scans. Only 2 of the 10 patients who underwent thyroidectomy had occult glandular foci of carcinoma, even among those with positive lymph nodes (patients 8, 9, and 10).

Although such a finding could be explained by incomplete sectioning of the thyroid gland specimen, the carcinoma is more likely to have developed de novo within the TDC instead of by metastasis or by local spread from a nearby thyroid carcinoma. If this hypothesis is true, excision of the TDC by the Sistrunk procedure may be adequate treatment of the carcinoma. The thyroid gland can be carefully palpated at surgery. If lesions can be detected, the patient should undergo lobectomy, and if the lesions are carcinomas, the patient should undergo total thyroidectomy. Patients should be followed up regularly for a prolonged period, because papillary carcinoma may grow slowly.[3] This tendency was shown by patient 9, in whom a neck mass with papillary carci-



Recommended algorithm for diagnosis of thyroglossal duct cyst carcinoma. TDC — thyroglossal duct cyst; FNA — fine-needle aspiration; MRI — magnetic resonance imaging; RAI — radioactive iodine.

noma developed nearly 5 years after the patient underwent the Sistrunk procedure and despite close follow-up; and by patient 10, in whom lung metastases developed nearly 5 years after treatment.

In summary, carcinoma is rare in TDCs, occurring in about 1% of cases, and is usually papillary. The condition generally occurs in young patients, but does not predominate in either sex. Preoperative diagnosis of malignancy is difficult, and therefore, the diagnosis is often made only after the TDC has been removed surgically.

An algorithm (see Figure) shows that in patients with apparent TDCs and no factors to indicate malignancy, we perform a Sistrunk procedure and carefully palpate the neck during the operation. If carcinoma is found incidentally after operation and no other lesions were noted during operation, no further treatment is planned, although regular monitoring for recurrence or metastasis is indicated after operation. Alternatively, with any cervical adenopathy, TDC, or thyroid mass, FNA is a cost-effective way to rule out carcinoma. If the FNA of the TDC is positive and palpation of the remainder of the neck is clinically negative, ultrasound or magnetic resonance imaging may locate undetected cervical metastasis or a primary lesion in the thyroid gland. A Sistrunk procedure alone is performed if the neck exploration and results of biopsy of suspicious lymph node or thyroid gland mass are negative. Frozen section confirmation of malignancy in the thyroid gland is followed by total thyroidectomy. Cervical metastasis is treated by modified neck dissection of the central or lateral compartments of the neck as appropriate. If FNA is negative, excision of the TDC by the Sistrunk procedure is indicated. Postoperative radioactive iodine scanning and subsequent ablation are indicated to eliminate persistent disease. Suppression with thyroid hormone can be used to prevent growth of occult tumor foci.

Long-term follow-up with clinical examination — with or without imaging studies — is mandatory.

ACKNOWLEDGMENTS — Charman Ibarra, CMT, assisted in preparing the algorithm. The Medical Editing Department, Kaiser Foundation Research Institute, provided editorial assistance.

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Ann Otol Rhinol Laryngol 110:2001

TRANSCANAL ATTICOADITOTOMY AND TRANSCORTICAL MASTOIDECTOMY FOR CHOLESTEATOMA: THE FARRIOR-OLAIZOLA TECHNIQUE REVISITED

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Conservative mastoid surgery in the management of middle ear cholesteatoma has been a subject of controversy for decades. Proponents of the combined-approach tympanoplasty for cholesteatoma do not entirely support the conservative approach in children, especially in the hands of an inexperienced surgeon. We describe here our experience with a modified technique of conservative mastoid surgery involving an atticoaditotomy and a separate simple mastoidectomy with lateral attic wall reconstruction (modified Farrior technique) that can be relatively safely used in the majority of cases of chronic squamous otitis media, even in children. This was a prospective analysis of the therapeutic effects and benefits of the technique used in 103 cases of cholesteatoma at Staffordshire General Hospital from 1991 to 1997. The follow-up was between 3 and 9 years. The study group consisted of 55 male patients and 48 female patients, including 28 children less than 16 years of age. The age ranges were 9 to 61 years in male patients and 10 to 48 years in female patients. After an audiological assessment, all patients underwent the described mastoid procedure, and some of them required revision surgery, mostly for a second look or for ossiculoplasty. The chief outcome measures used were complete eradication of disease, incidence of disease recurrence, and restoration of hearing. The steps of the procedure, its pros and cons, and the results of the series are presented. Also discussed is an overview of the literature pertaining to intact–canal wall mastoid surgery. Of the 103 patients, only 3 had a recurrence of disease, and 9 patients showed no hearing improvement. We conclude that this modified technique has several advantages over the previously described mastoidectomy procedures. It is a relatively simple and effective technique that can be performed even in children.

KEY WORDS — cholesteatoma, closed-cavity mastoidectomy, intact–canal wall tympanoplasty, ossiculoplasty.

INTRODUCTION

The management of active squamous otitis media both in adults and in children has been a real challenge to the adept otologist over the past few decades. Before the 1950s, the only surgery that was popularly recommended and used for chronic suppurative otitis media was radical or modified radical mastoidectomy.[1] The purpose of these operations was to render the ear safe by complete eradication of the disease and exteriorization of the cavity for long-term management.[2]

There is no controversy today about the need for surgery in most cases of aural cholesteatoma, regardless of the age of the patient. A subject debated endlessly is the choice of the surgical technique: open versus closed mastoidectomy. The arguments used by supporters of the open-cavity procedure are based on the conviction that there is a higher incidence of recurrent or residual cholesteatoma after closed procedures. However, proponents of the closed-cavity procedure, ie, combined-approach tympanoplasty (CAT), have repeatedly reinforced the fact that hearing results are extremely disappointing with the unphysiological conditions that exist after the creation of an open mastoid cavity.[3,4] Added to the problems of deafness and disability with an open cavity is the loss of a trouble-free external canal with a self-cleaning function, which leads to the collection of debris and hence physician dependence.

Many otologists have made avoidance of the mastoid cavity a primary goal of ear surgery. Skillful otologic surgeons have designed innovative procedures that preserve the posterior canal wall, thus avoiding the mastoid recess.[3,4] The transition from radical and modified radical mastoidectomy to the intact–canal wall tympanoplasty with mastoidectomy has been slow but gratifying. Ever since the procedure of CAT was first described in the late 1950s, the philosophy of closed-cavity mastoidectomy has evolved, amid much uproar and skepticism in otolaryngological circles.

Although the technique has received some critical acclaim in the past 3 decades, there are a few shortcomings of the procedure that are acknowledged by

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Presented in part at the XVI World Congress of Otorhinolaryngology, Head and Neck Surgery, Sydney, Australia, March 2-7, 1997, and presented at the 6th International Conference on Cholesteatoma and Ear Surgery, Cannes, France, June 29–July 2, 2000.

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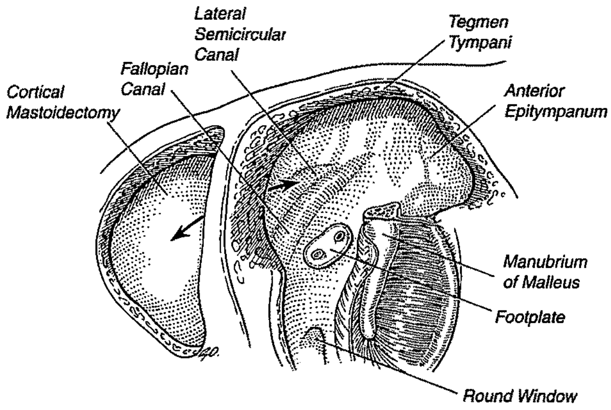

Fig 1. Schematic diagram of right ear shows atticoaditotomy and simple mastoidectomy.

authorities in the field. Hence, the procedure has seen a few modifications in the past 2 decades.[5-8] The procedure is technically more difficult to perform than the open-cavity surgery, for many individuals. Also, there is a well-accepted planned second stage for reexploration of the mastoid antrum for residual cholesteatoma and an ossiculoplasty, if need be. Most authors emphasize the need for careful selection of cases of limited cholesteatoma and the surgeon's essential expertise and experience in middle ear and mastoid surgery. It is also known that many supporters of canal wall–up surgery do not favor the use of this procedure in children.

We have developed a modified technique of intact–canal wall tympanoplasty (modification of the Farrior[7] and Olaizola[8] procedures) that overcomes most of the disadvantages of CAT listed above. The procedure essentially involves an atticoaditotomy, a simple mastoidectomy with a minimal canal wall reconstruction, and a tympanoplasty, and can be used relatively safely even in children. We present here 103 patients treated with this technique, including 28 children, with a 3- to 9-year follow-up range. The steps of the surgery and the advantages and disadvantages are discussed in detail.

PATIENTS AND METHODS

The study was undertaken at Staffordshire General Hospital in the West Midlands of England, which is a large District General hospital with about 400 beds. All patients who presented to Mr Reddy's practice from January 1991 and received a diagnosis of cholesteatoma have been included in the study irrespective of their ages. Those patients who had already had previous surgery for atticoantral disease and were being followed up were excluded from the study.

From 1991 to 1997, a total of 103 patients with chronic squamous otitis media were seen and underwent the described technique. This included 75 adults and 28 children, of whom 55 were male and 48 were female. The mean age for male patients was 39 years, and that for female patients, 32 years. The follow-up range was 3 to 9 years (12 cases, 9 years; 18 cases, 8 years; 17 cases, 7 years; 11 cases, 6 years; 12 cases, 5 years; 19 cases, 4 years; and 14 cases, 3 years). The waiting time for surgery was between 2 and 5 months. All of the patients underwent an audiological assessment on their first visit and on the day of surgery. Of the 103 cases, 60 were attic cholesteatomas and 43 were posterior-segment cholesteatomas. There were no extracranial or intracranial complications.

All procedures were performed under general anesthetic and local infiltration of lidocaine hydrochloride with epinephrine. A modified Bennett type of endaural incision was used. After a temporalis fascia graft was obtained, the mastoid cortex was exposed by lifting back a conchomeatal flap, and bone chips were harvested with a gouge and hammer or a microdrill from the mastoid fossa.

A classic tympanomeatal flap elevation was followed by meticulous bone drilling, which included an initial posterior canal widening and atticotomy extended to an aditotomy and a posterior simple mastoidectomy as shown in Fig 1. Delivery and excision of the cholesteatoma in toto from behind forward with removal of the incus and amputation of the malleus head followed, when required for disease clearance. The key areas in the middle ear cleft that were meticulously explored and cleared of disease included the facial recess, sinus tympani, incudal fossa, anterior epitympanum, hypotympanum, eustachian tube orifice, and mastoid air cell system. Total clearance, especially in the regions of the facial recess and sinus tympani (posterior mesotympanum), was confirmed with the use of Buckingham mirrors and angled otologic endoscopes where necessary, after widening of the bony meatus. When the middle ear mucosa was unhealthy or abraded, it was decided to place a very thin strip of silicone rubber on the medial tympanic wall to include the medial attic and aditus walls. This was done to prevent adhesions, create an adequate middle ear space with improved aeration of the middle ear cleft, and promote reepithelialization underneath.

The lateral attic and aditus walls were reconstructed with the previously harvested bone chips sculptured to shape to maintain atticoantral patency (Fig 2). In certain cases, especially in children in whom difficulty in harvesting bone chips was encountered, tragal cartilage with perichondrium was used instead.

A primary ossiculoplasty was performed in most of the cases with a disease-free sculptured autologous

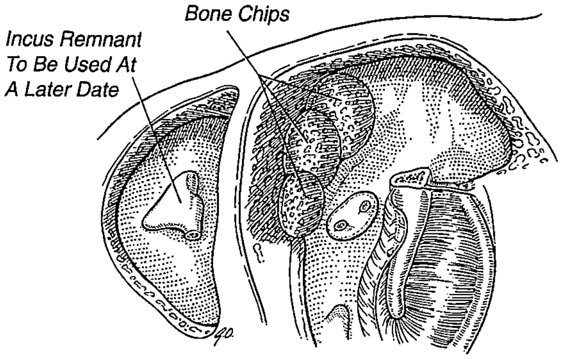

Fig 2. Schematic diagram of right ear shows scutumplasty with bone chips; also shows autologous incus stored in mastoid bowl for future use when ossiculoplasty is staged.

incus for a neck-of-malleus to head-of-stapes assembly. If a sufficiently large, healthy incus was unavailable, a hydroxyapatite partial or total ossicular replacement prosthesis was used. Selective use of tragal cartilage for ossicular chain reconstruction was done when neither of the above methods was possible.

Ossiculoplasty was staged in some cases of extensive infection, extensive granulation, or extensive cholesteatoma, especially in children. In many of these cases, the autologous incus was saved in the patient's simple mastoid bowl for future use (Fig 2). A second-stage procedure was always performed within 2 years and not before 6 months after primary surgery. Fibrin glue was used when required to stabilize the bone chips and the reconstructed assembly.

The tympanic membrane was grafted with temporalis fascia by the underlay technique. The canal was packed, and the wound was closed in layers and dressed with pad and bandage. The patients were seen 3 weeks after operation, when the canal packing was removed, and antibiotic steroid ear drops were instilled for 2 weeks.

Further follow-up included a second visit after 6 weeks for a canal inspection and clearance, and a third visit at 3 months, when the first postoperative audiogram would be done. Each patient was regularly followed up, quarterly in the first year and half-yearly in the subsequent years.

RESULTS

Sixty of the 103 patients had attic cholesteatomas, and 43 had posterior-segment cholesteatomas. Twenty-seven of the patients had bilateral disease, and 15 of these had a previously dry mastoid cavity on the opposite ear. Twelve others were awaiting surgery for the opposite ear. (All 12 have had surgery for the second ear since 1997 by this specific technique.)

The lateral attic and aditus wall reconstruction, popularly called "scutumplasty," and part of the bony annulus reconstruction were done in most of the cases with sculptured bone chips (Fig 2), and it was noted that usually about 3 bone chips suffice. Bone chips were used in 89 cases (71%), and cartilage (tragal or conchal) was used in 14 (29%). The bone chips or cartilage were placed with their concavity medially to allow mastoid aeration.

TABLE 1. OSSICULOPLASTY TECHNIQUES

Primary	84 (81.5%)	Autologous incus	52
		HA	24
		Tragal cartilage	8
Second-stage	19 (18.5%)	Autologous incus	8
		HA	6
		Septal cartilage	5

HA — hydroxyapatite (total or partial ossicular replacement prosthesis).

Ossiculoplasty (Table 1) was performed primarily in 84 cases (81.5%) and as a second stage in 19 cases (18.5%). Incus transposition was performed primarily in 52 cases, and in 8 cases, the autologous incus stored in the mastoid bowl was used at a second stage. In cases in which the superstructure of the stapes was absent and/or it was decided that the autologous incus was insufficient or unhealthy, a hydroxyapatite ossicular replacement prosthesis (total or partial) was used (24 primary and 6 second-stage). In certain cases, selective use of cartilage was made, and this included tragal cartilage in 8 cases in the first stage and septal cartilage in 5 second-stage cases.

The postoperative hearing improvement in most of the patients has been quite gratifying. Table 2 shows

TABLE 2. PREOPERATIVE AND POSTOPERATIVE AIR-BONE GAPS

Preoperative Air-Bone Gap Bins (dB)	*No. of Patients*	*Postoperative Air-Bone Gap Bins (dB)*	*No. of Patients*
Primary reconstruction			
0-10	0	0-10	0
11-20	5	0-10	5
21-30	19	0-10	3
		11-20	12
		21-30	4
>30	60	0-10	49
		11-20	5
		21-30	3
		>30	2
Staged reconstruction			
0-10	0	0-10	0
11-20	2	0-10	2
21-30	6	0-10	5
		11-20	1
>30	11	0-10	7
		11-20	2
		>30	2

Level 1 data summary as recommended by Committee on Hearing and Equilibrium.[9]

TABLE 3. GLASGOW BENEFIT PLOT

Preoperative Group	*Number (n = 103)*	*Postoperative Category*			
		a	*b*	*c*	*d*
1	76 (4 no gain)	72	NA	NA	NA
2	16 (2 no gain)	NA	9	4	1
3	11 (3 no gain)	NA	7	1	3

Technique of Glasgow Benefit Plot is from Browning et al.[10] Table shows number of patients in each preoperative impairment group as they fall into each postoperative category. To be read with Fig 3.
NA — not applicable to that specific group.

level 1 data summarized as recommended by the Committee on Hearing and Equilibrium.[9] The data presented show the preoperative air-bone gap bins (average for 0.5, 1, 2, and 3 kHz) and their respective conversions after operation with the primary and second-stage ossiculoplasties. It can be seen that technical success was achieved in 94 cases (91.2%; 77 primary and 17 staged). There was no change in hearing in 9 cases (8.8%; 7 primary and 2 staged).

Discussing hearing gains by means of the popular Glasgow Benefit Plot as described by Browning et al[10] in 1991 is quite topical. It is beyond the scope of this paper to discuss in profound detail the various aspects of the scheme or present the plots for all 103 cases. In Table 3, an attempt is made to summarize the benefits by applying the preoperative "groups" and the postoperative "categories" to each of the 103 cases. This Table is to be read in conjunction with Fig 3,[10] which is self-explanatory. The preoperative hearing impairment groups are classified as group 1 (unilateral hearing loss), group 2 (bilateral asymmetric hearing loss), and group 3 (bilateral symmetric hearing loss). The postoperative hearing categories include category a (bilateral normal hearing), category b (unilateral normal hearing), category c (operated ear improves and becomes the better-hearing ear), and category d (symmetric hearing loss).

Of the 76 patients in group 1, 72 showed hearing improvement that fell into category a. Four of these patients showed no gain in hearing. Sixteen patients were in group 2, of whom 9 improved to normal hearing on the operated side; in 4 cases, hearing improved only to make the operated side the better-hearing ear. In 1 case, hearing improved only marginally, for an outcome of symmetric hearing loss. Two patients in group 2 showed no change in thresholds. Of the 11 patients in group 3, 7 showed remarkable improvement that rendered the operated ear normal, and in 1 case, the operated ear became the better-hearing ear. Three cases in this group showed no change in thresholds and hence remained as bilaterally symmetric deaf ears.

It is very obvious that with this system, "technical success" need not necessarily coincide with "patient benefit."

Three patients had recurrence of cholesteatoma. Two, upon revision, showed bone chip disintegration (true recurrence with retraction pockets), and 1 showed subluxation of cartilage with skin trapped behind it (residual cholesteatoma); in addition, necrosis of the tragal cartilage struts used for ossiculoplasty was found in all 3. One of these patients underwent revision in the form of a modified radical mastoidectomy, and the other 2 underwent revision scutumplasty with conchal cartilage. All 3 of these patients have done well since their revision surgeries.

Nine patients had no change in hearing after ossiculoplasty, and these included 7 patients with primary ossicular chain reconstruction and 2 with staged procedures. On reexploration, 5 of these patients had extrusion or displacement of the ceramic prosthesis (3 primary and 2 second-stage), and 4 had cartilage necrosis or absorption (all primary; 3 had recurrent cholesteatoma). Most revisions and reexplorations were performed between 9 and 15 months after surgery. In all of these cases, revision ossiculoplasty was

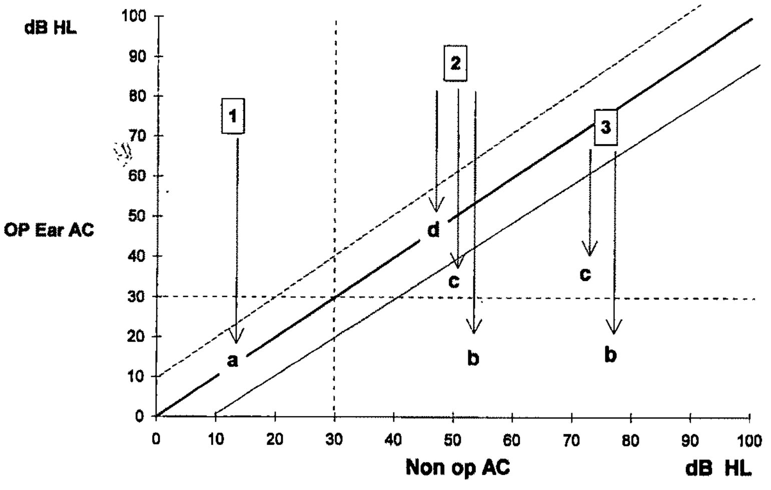

Fig 3. Glasgow Benefit Plot.[10] Basic scheme shows preoperative groups 1, 2, and 3 and postoperative categories a, b, c, and d (see text under Results). OP — operated; AC — air conduction; HL — hearing level.

performed with a hydroxyapatite prosthesis, and the authors are pleased to note that these cases have improved from group 1 (n = 4), group 2 (n = 2), and group 3 (n = 3) to postoperative categories a, b, and b, respectively (data not in Tables).

DISCUSSION

Until 1958, the surgical management of acquired cholesteatomas consisted essentially of exteriorization of cholesteatomas, resulting in open cavities with their attendant problems. Open cavities were subject to recurrent infections, and the patients needed lifelong medical attention for cavity clearance and hence had restrictions on social activity. The CAT, when introduced, was a welcome alternative to management of squamous otitis media and generated a great deal of interest among dedicated otologists.[1,3] The initial enthusiasm for the technique gradually died in the late 1970s and early 1980s, as a number of surgeons were disillusioned by the long-term results.[11-13] The technique is extremely individualized and requires experience and skill on the part of the surgeon. This fact is remarkably well elucidated by the enormously variable recurrence rates reported in the literature: from 3% to 62%.[1-3,11,12,14-19]

The CAT involves a standard procedure consisting of an extended cortical mastoidectomy with exposure of the attic as an outside-in technique together with removal of the incus, the head of the malleus, and any bony shelf in the attic. A posterior tympanotomy is performed in all patients, and one may choose to reconstruct large outer attic wall defects with bone or cartilage. No attempt is made at any primary reconstruction of the ossicles, irrespective of the health of the middle ear and its mucosa. The procedure is always staged, and a second look is planned in 6 months to 1 year. It is generally agreed that the procedure is technically difficult to perform, is time-consuming, and demands expertise and skill on the part of the surgeon. It is definitely not for the general otolaryngologist! It would appear that the clearance of squamous epithelium, especially in the regions of the anterior attic, incudal fossa, and tympanic sinus, is less than satisfactory with the CAT.[5,6] One study of this technique lists the various causes of failure encountered: facial ridge–tympanic membrane adhesions, severe silicone reactions, incomplete removal from the sinus tympani due to a grossly overhanging facial nerve, large dermoids, and total eustachian tube obstruction.[20]

The Tos[5,6] technique consists of a postaural incision to perform a transcanal and transmastoid approach that includes a simple mastoidectomy and an atticotomy sparing a bridge of bone between the attic and the mesotympanum together with a limited posterior tympanotomy. The author describes an "otosclerosis drilling" of the posterior bony annulus and the posterosuperior canal wall overhang that is essential for the complete clearance of the posterior mesotympanum, specifically, the sinus tympani. This region can in no way be cleared satisfactorily by a posterior tympanotomy alone. The author, in the case of an intact ossicular chain, does not recommend a posterior tympanotomy.[5,6] A primary ossiculoplasty is always the objective, and the scutumplasty is done with fascia, cartilage, or bone. Fascia used in the initial procedures to inspect the attic for any recurrent cholesteatoma was unsatisfactory, because of frequent retractions, and hence the author used septal cartilage or cortical bone.[5,6] This technique is no doubt simpler than the CAT, and some of the salient features have been incorporated in our technique. We believe that there is not much to be gained by keeping a thin bridge of bone of the scutum that could easily erode away, making the supports unstable. It could incite new bone formation, incite adhesion formation, leave behind shreds of epithelium, and, above all, make the technique more difficult to perform.

The Farrior[7] technique involves a wide enlargement of the bony ear canal, even as far as the middle fossa dural plate, and a transcanal anterior atticotympanotomy to safely clear the anterior attic. Removing the posterior bony annulus exposes the posterior mesotympanum, including the sinus tympani. A separate cortical mastoidectomy is performed, and this complete dissection creates a widely opened middle ear cavity, similar to that of a modified radical cavity, but still preserves the posteriorly displaced bony ear canal wall, thus creating a closed mastoid cavity. Farrior also recommends the use of the malleus head and a piece of bone in the attic to maintain a bony annulus, and a piece of temporalis muscle in the facial recess area to prevent retractions. Whereas the Farrior technique attempts repneumatization of the mastoid antrum and the attic, the Olaizola[8] technique excludes the same by using a large piece of cartilage for obliteration, the so-called "antrum exclusion and attic elimination on-demand technique." Our technique approaches the problem through an endaural transmeatal tympanotomy with an atticoaditotomy and a simple cortical mastoidectomy. We have found that this technique gives complete exposure of any type of cholesteatoma (attic, atticoantral, mesotympanic, and tympanomastoid) and its various extensions to the so-called "blind" corners already mentioned in Patients and Methods. We do not believe the facial recess needs to be cleared by a separate posterior tympanotomy. Annulus drilling and the use of the traditional Buckingham mirrors and angled telescopes as required[21] for inspection would ensure complete clearance in the posterior mesotympanum. Sculptured bone chips, harvested previously from the exposed bone before drilling, are excellent for lateral attic wall reconstruction. Although this seems like a limited open cavity, it is believed that with the excellent scutumplasty, re-aeration of the aditus and antrum is achieved and the middle ear configuration is brought back as nearly as possible to normal, both anatomically and functionally. If the posterior mastoid bowl is not ventilated, the situation is then similar to that of the Olaizola technique.

We recommend the use of thin silicone rubber on the medial attic and mesotympanic walls only in cases of extensive surgical mucosal trauma and excessive inflammation. Thin silicone rubber tends to result in very minimal mucosal reaction and is excellent in preventing adhesions. Thick silicone rubber incites a very strong reaction, which may result in failure of ossiculoplasty, graft rejection, and a severe infection. Thick silicone rubber, in our experience, has many times formed a large lump with a pseudocapsule around it, inciting a foreign body reaction.

There were only 3 cases of failure with our technique (2.9%), in the form of recurrent cholesteatomas. The problem of "recidivism" with the CAT has been addressed in great detail by Yanagihara et al.[22] The authors discuss 3 types of solutions to prevent recurrence at a planned second-stage operation, ie, type S1, only ossiculoplasty; type S2, ossiculoplasty and scutumplasty; and type S3, ossiculoplasty, scutumplasty, and mastoid antrum obliteration. Although the rate of recidivism was significantly reduced, deep retraction pockets developed in 15% of adults and in 23% of children. The incidence of deep retraction pocket formation was lowest in the adults with type S1 operations and highest in the children with type S3 operations. We have had no problems with deep retraction pockets in our series so far. It has been nearly 4 decades since the CAT was introduced, and to date, the technique is unacceptable in children to a significant body of otologists. Cholesteatoma in children presents special surgical problems because of its fast and invasive growth and high recurrence rate. Wullstein[23] has referred to the fact that in the early phase of development of the temporal bone, the squamous matrix and the perimatrix find favorable requirements for rapid spread, as the air-filled spaces have not reached adult size. There is also a second phase of bone growth that is slower and is required for the mucociliary system to develop its immunobiological properties; hence, childhood in this sense really lasts until about 16 years.[23] The results of surgery for cholesteatoma in children have been so variable[6,24] that one may surmise that the approach needs to be individualized with each case. In our experience, 1 of the 28 children under 16 years had recurrent disease, 3 needed staging for ossiculoplasty, and 2 needed revision tympanoplasty (including the child with recurrent cholesteatoma).

It can be understood that the primary aims of any intact canal wall surgery for cholesteatoma are complete disease clearance and reestablishment of a trouble-free external canal with its self-cleaning function. However, we believe that the second goal of hearing restoration in these ears deserves better attention than it has received so far. We believe that there is no harm done and not much to lose by performing primary ossiculoplasty in most cases of retraction pocket cholesteatomas, which are usually dry and uninfected. Ossicular chain reconstruction should not be performed in secondary acquired extensive cholesteatomas with profuse granulations. In our experience, the best results have been with the use of an autologous incus, and the ceramic prosthesis is second-best.

Septal cartilage ossiculoplasty deserves special mention. All 5 patients in whom the technique was used in a second-stage procedure have done extremely well. The relative firmness of this cartilage perhaps prevents absorption and necrosis — unlike tragal cartilage.

For presenting the hearing gains of any hearing restoration surgery, the Glasgow Benefit Plot has gained tremendous popularity and support. The accepted notions of "technical success" and "patient benefit" hence become very important clinical outcome measures. As can be seen in Tables 2 and 3, technical success may not always equate to patient benefit. As the Glasgow Benefit Plot is a logical method of reporting hearing preservation or restoration, no attempt has been made to apply the conventional statistical packages to our data. In 9 cases, revision ossiculoplasty has yielded favorable results, as alluded to previously.

CONCLUSION

We believe that this modified technique of intact-canal wall tympanoplasty is a relatively simple and safe technique that can be performed in most cases of cholesteatoma. It has been our experience that the technique is quite effective even in children, although the numbers have been small. We also believe there is a case for primary ossiculoplasty in most patients, and that the Glasgow Benefit Plot and Committee of Hearing and Equilibrium guidelines are two effective methods for analysis of hearing preservation or restoration.

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FAILURE OF GROMMET INSERTION IN POST-IRRADIATION OTITIS MEDIA WITH EFFUSION

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Nasopharyngeal carcinoma (NPC) survivors with post-irradiation otitis media with effusion (OME; 100 ears) were divided into 2 groups. One group underwent grommet insertion, and the other group was treated by repeated myringotomies plus aspiration to evaluate the outcome of the OME. Computed tomography, magnetic resonance imaging, audiometry, and local checks of the ear, nose, and nasopharyngeal fields were performed to evaluate the sinus and middle ear conditions over a long-term (more than 10 years) follow-up period. The results in these NPC survivors with OME indicated that the prevalence of middle ear complications in the myringotomized group (33%) was less than that in the grommeted group (90%). Grommets alone cannot eradicate inflammation outside the middle ear cavity, but rather, they can aggravate it by superinfection. We therefore recommend that controlling the radiation-induced inflammation in areas such as the ears, nose, sinuses, and nasopharynx is most important. In conclusion, post-irradiation OME should be treated in a different way from conventional OME. Restated, grommet insertion is contraindicated in post-irradiation OME.

KEY WORDS — grommet, irradiation, myringotomy, nasopharyngeal carcinoma, otitis media with effusion.

INTRODUCTION

Post-irradiation otitis media with effusion (OME) may be a disease entity different from conventional OME. Radiotherapy for nasopharyngeal carcinoma (NPC) covers an area from the skull base to the clavicle,[1,2] and hence, the eustachian tube,[3-7] middle ear,[8] nose, sinuses, parapharynx, and oropharynx are included in the radiation field. Thus, treatment complications of these organs in the radiation field are unavoidable.[3] Grommet insertion was once thought to be the obvious treatment method for OME in NPC patients.[9] However, a long-term follow-up study of NPC survivors with grommet insertion does not support this assumption.[4] On the contrary, more and more cases of post-irradiation OME in patients who underwent grommet insertion resulted in chronically discharging ears.[10,11] Why is it that grommet insertion could be beneficial in conventional OME, but not in post-irradiation OME? In this study, we sought to evaluate the efficacy of grommet insertion and that of myringotomy alone in the outcome of post-irradiation OME in long-term survivors of NPC.

PATIENTS AND METHODS

This retrospective study consisted of 67 long-term (more than 10 years) survivors of NPC, of whom 36 were men and 31 were women. Their ages at NPC diagnosis ranged from 20 to 76 years, with a mean of 46 years. The mean interval after the completion of radiotherapy was 11 years. These patients had 100 ears with post-irradiation OME during the past decade. They were managed incidentally and could be

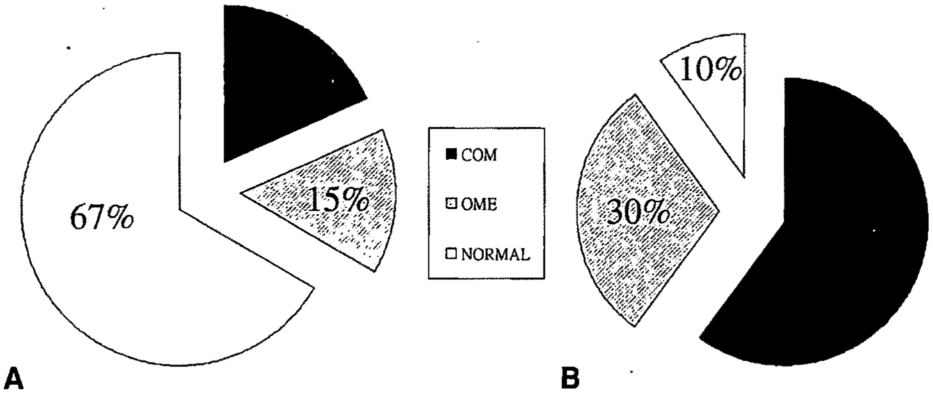

Fig 1. Prevalence of middle ear complications in **A)** myringotomized ears (n = 60) versus **B)** grommeted ears (n = 40).

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Presented at the Second International Symposium on Middle Ear Mechanics in Research and Otosurgery, Boston, Massachusetts, October 21-24, 1999.

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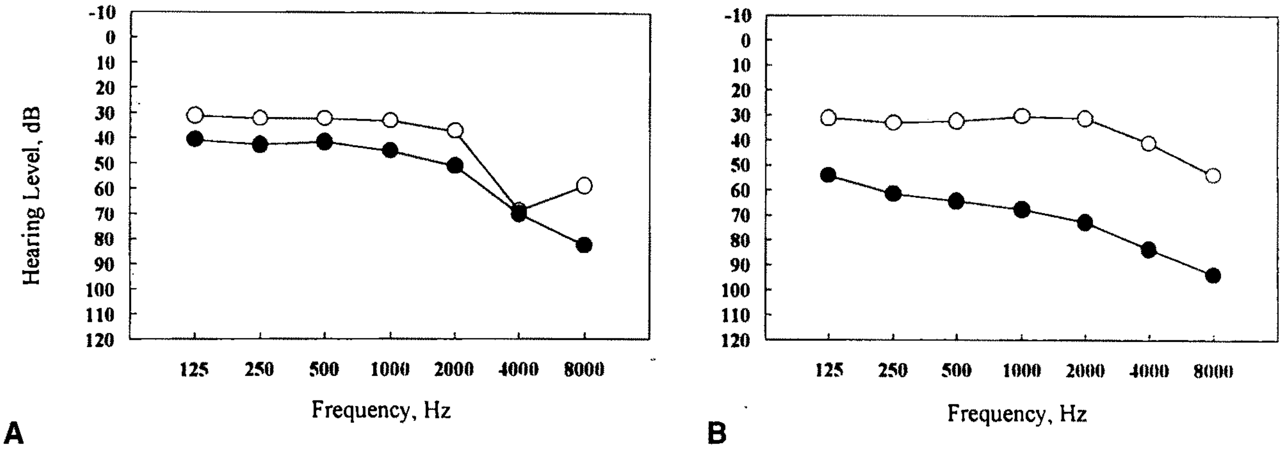

Fig 2. Mean hearing levels for air conduction in A) myringotomized ears versus B) grommeted ears before irradiation (hollow circles) and 10 years after irradiation (solid circles). There is significant hearing deterioration in grommeted group ($p < .05$), but not in myringotomized group ($p > .05$).

divided into 2 groups: 40 ears underwent Teflon grommet insertion (G group), and 60 ears were treated by repeated myringotomies plus aspiration (M group).

The G group consisted of 11 men and 16 women, ranging in age from 32 to 72 years (mean, 54 years). The M group consisted of 25 men and 15 women, ranging in age from 30 to 86 years (mean, 56 years). Both groups received a mean radiation dosage of 75 Gy. Each patient underwent computed tomography or magnetic resonance imaging, audiometric evaluation, and examination of the ears, nose, and nasopharynx in both preirradiation and post-irradiation periods.

RESULTS

Of the M group (n = 60 ears), 11 ears (18%) developed chronic otitis media (COM; with discharge), 9 (15%) had OME, and 40 (67%) were free of disease. Of those that underwent grommet insertion, 24 ears (60%) developed COM, 12 (30%) had OME, and only 4 (10%) were free of disease (Fig 1). In other words, 90% of the grommeted ears had middle ear complications such as COM or persistent OME, compared to 33% of the myringotomized ears — a significant difference (Student's *t*-test, $p < .05$).

There was no significant difference ($p > .05$) in mean hearing levels for air conduction and bone conduction between the pre-irradiation period and the 10 years' post-irradiation period for the group of myringotomized ears. However, in grommeted ears, there was deterioration of hearing at 10 years after irradiation, for both air conduction and bone conduction (Figs 2 and 3).

The 100 ears were redivided into 2 groups to further evaluate the relationship between radiation dosage and middle ear complications. Group A consisted of those with radiation dosages equal to or less than 70 Gy (n = 50 ears), and group B consisted of those with radiation dosages of more than 70 Gy (n = 50 ears). In group A, 46% of the ears had COM or OME

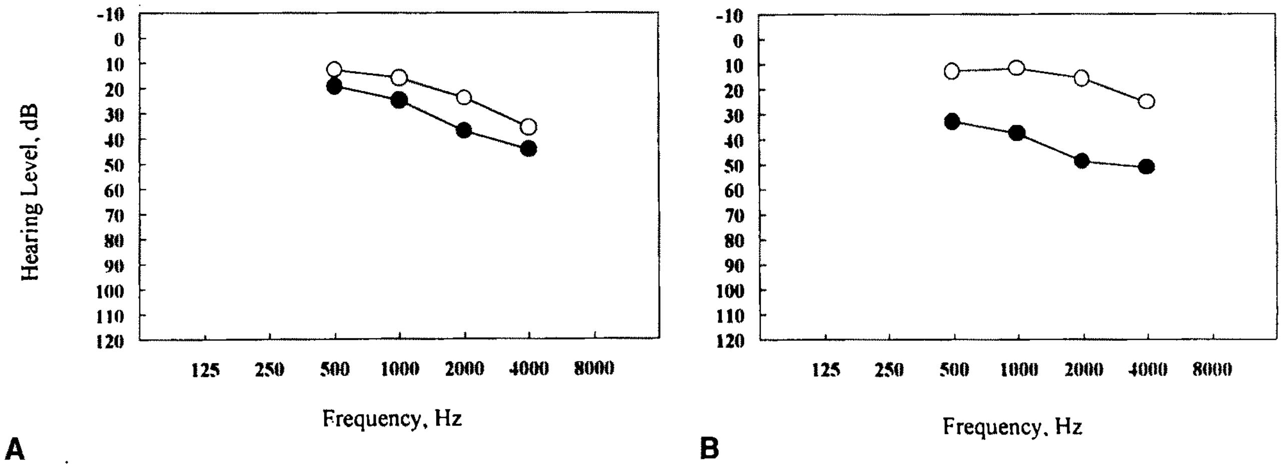

Fig 3. Mean hearing levels for bone conduction in A) myringotomized ears versus B) grommeted ears before irradiation (hollow circles) and 10 years after irradiation (solid circles). Hearing deterioration is noted in grommeted group ($p < .05$), but not in myringotomized group ($p > .05$).

at 10 years after irradiation, compared to 58% of the ears in group B (no significant difference; p > .05).

DISCUSSION

According to our previous report,[12] post-irradiation sudden deafness differs from idiopathic sudden deafness. Similarly, according to the present study, post-irradiation OME seems to differ from conventional OME in 2 ways. First, previous radiotherapy to the temporal bone results in post-irradiation OME. Second, grommet insertion is beneficial in conventional OME, but not in post-irradiation OME.

Grommeted ears in post-irradiation OME fare worse than myringotomized ears when middle ear complications or hearing status is considered over a long-term follow-up period. In long-term survivors of NPC, 90% of grommeted ears developed middle ear complications. However, only 33% of myringotomized ears developed such complications (Fig 1). How can we explain this finding?

In cases of conventional OME, grommets may force the opening of the eustachian tube and drain the middle ear effusion via the eustachian tube.[10] However, in post-irradiation OME, infectious foci from all radiation-exposed areas, such as sinusitis or nasopharyngitis, may ascend via the opening of the eustachian tube into the middle ear cavity by reflux, causing superinfection of the middle ear.[4,10] Therefore, chronic discharge can often be found in a grommeted ear of a long-term survivor of NPC. Since both tubal obstruction and inflammatory reaction are responsible for post-irradiation OME,[10] repeated myringotomy plus aspiration may drain the middle ear effusion and temporarily reduce the inflammatory reaction of the middle ear. One long-term follow-up study reveals that tubal obstruction can be relieved by the development of a patulous tube 10 years after irradiation.[5] Thus, once tubal function is recovered, the middle ear experiences fewer complications.

We therefore recommend that protecting those areas inflamed by radiation, such as the ears, nose, sinuses, and nasopharynx, is most important. Grommet insertion alone cannot eradicate inflammation outside the middle ear cavity, and may aggravate it by superinfection. In conclusion, post-irradiation OME and conventional OME should be treated differently; that is to say, grommet insertion is contraindicated in post-irradiation OME.

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DEMONSTRATION OF INTRAVITAL MICROFISSURES IN UNDECALCIFIED PLASTIC-EMBEDDED TEMPORAL BONES WITH THE PRESTAINING TECHNIQUE

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Microfissures in the human otic capsule have been observed since the start of the century, but it was Otto Mayer, in 1930, who first realized that some of them were of intravital origin and not just processing artifacts. Since then, a small number of publications, based on decalcified temporal bones, have mostly confirmed his findings. With the introduction by Frost in the late 1950s of the undecalcified bone technique and the bulk staining technique for peripheral bones, a method was developed and refined for identifying even very small intravital microfissures (microdamage). Bulk staining of undecalcified otic capsules has not yet been used to verify the findings from the previous decalcified specimens. The present report presents our experience with the pertinent techniques, and suggests modifications and shortcuts pertinent to temporal bone research. Both large and tiny microfissures of intravital genesis are demonstrable within bulk-stained undecalcified human otic capsules. The importance of microfissures in the petrous bone in the causation of otosclerosis and perilymphatic leakage has long been discussed, and the present techniques may advance our understanding of these pathological conditions.

KEY WORDS — artifact, bulk staining, fluorescence microscopy, microdamage, microfissure, otic capsule, perilabyrinthine bone, prestaining, undecalcified technique.

INTRODUCTION

A vigorous controversy was started in 1930, when Otto Mayer[1,2] introduced the notion of in vivo microfissures in the otic capsule. On the basis of findings in the cells, vessels, and connective tissue within the lumen of some smaller cracks related to the perilymphatic spaces, he assumed them to be intravital in origin, and although their cause remained speculative, they apparently did not result from gross external head injuries. Mayer[2] proposed several explanations for their origin, among which were pressure from the brain weight and the trivial but abundant vibratory stresses from the temporomandibular joints. He was vigorously opposed by Wittmaack,[3] who disputed the intravital origin of the cracks and claimed them to be artifacts. In the following years, these opposite opinions were argued by adherents of Mayer's opinion[4-8] and of Wittmaack's.[9-11] The dispute may have been enhanced by the wish of Mayer to explain otosclerosis as a callus formation, in response to the claimed intravital fissures. However, the latter hypothesis did not find any support, primarily because of differences in prevalence and predilection.[3,9,12] After World War II, an apparent armistice on the matter is seen, the few articles on the topic tacitly accepting the existence of intravital intracapsular microfissures without gross traumatic origin, and primarily debating their topographic distribution and possible etiologic factors.[13-22]

In 1958, Frost[23] introduced the cutting/grinding technique for the preparation of undecalcified bone specimens, making it possible to avoid the shrinkage from decalcification and to preserve the information hidden in the mineralized ground substance of bone, such as mineral density and bone-seeking fluorochromes (ie, tetracyclines),[23] and this method has already been used within the temporal bone research field.[22,24-27] In 1960, inspired by the techniques used to verify fatigue damage in metals, concrete, and other building materials, Frost[28] launched the bulk staining technique to prove the intravital origin of the microscopic cracks seen in undecalcified histologic specimens. Before any handling of the specimen, the whole bulk of tissue was immersed in 1% basic fuchsin dissolved in ethanol for a time sufficient to stain it throughout, and after this combined staining and fixation, routine procedures for undecalcified bone preparation such as cutting and grinding could take place. By this method, Frost found stained, as well as unstained, microscopic fractures in the pars compacta of various bones and concluded that the stained ones were intravital and the unstained were artifacts, although he acknowledged that some genuinely intravital cracks may not take up stain and thus could be

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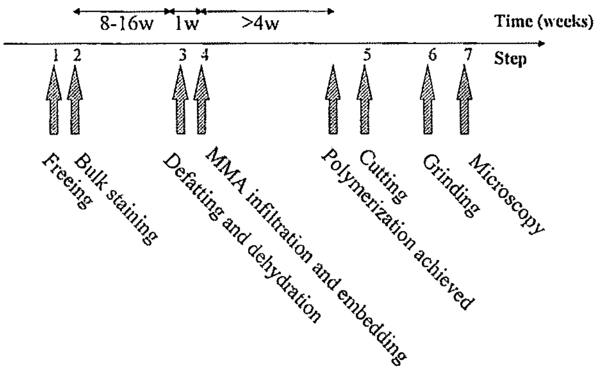


Fig 1. Schematic presentation of various processing steps in bulk staining procedure, with approximate durations given. MMA — methyl methacrylate.

erroneously classified as artifacts. He proposed several conditions for accepting a stained crack as intravital: first, the crack had to be stained throughout the depth of the section; second, the crack had to open visibly onto the surface; and third, the crack should not leak stain into the bony substance in the walls of the crack. Frost considered these slender histologic cracks to be fatigue failure due to daily life's trivial (physiological) traumas to the bones, parallel to the fatigue cracks seen in metals and building materials, and he denoted them fatigue microdamage. Later, this interpretation was examined and corroborated.[29,30]

This bulk staining technique has never been used to verify microfissures in undecalcified human temporal bone specimens. The principal aim of this study was to substantiate the existence of capsular microdamage and to develop a possible standard procedure for temporal bone preparation. This technique may enhance our understanding of the etiology and pathogenesis of otosclerosis and of perilymphatic leakage.

MATERIAL AND METHODS

The material consisted of 136 otic capsules from 136 individuals, at least 40 years of age, procured from routine autopsies in which the skull was to be opened for neuropathologic examination. No selection of the material was made before analysis on the basis of known disease or sex. The main purpose of all steps in the preparation procedure was to reveal well-delimited, thoroughly stained microfissures within an unstained bone substance. The preparation process is shown diagrammatically in Fig 1. The specimens were ground to 150-µm thickness ("coarse" level) and analyzed by epifluorescence microscopy (see below). To validate the use of "coarse" slices, a comparison to specimens with slices ground to "raw" (250 µm, n = 13) or "fine" (50 µm, n = 17) levels was undertaken (Fig 2). Further, as controls, 15 specimens had consecutive analyses at all 3 levels and 11 specimens were embedded directly in fluorescent dye.

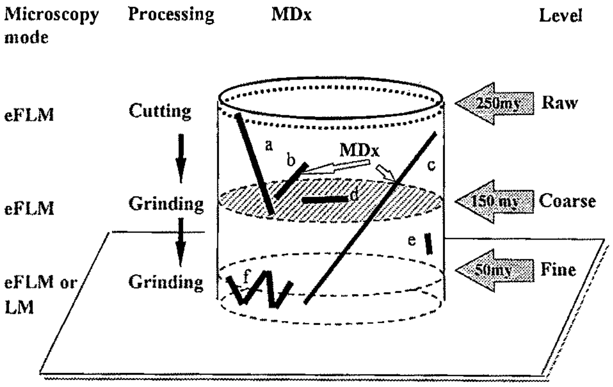


Fig 2. Diagram of various surfaces exposed during grinding of cut undecalcified slice. Intravital bulk-stained microcracks (MDx) are shown as lines a through f. Only larger cracks (a, c) may be represented at several levels. Small cracks may be eliminated by grinding (e), or may be missed because of their orientation parallel to surface of inspection (d). Fine grinding may lead to separation of visible projections of continuous cracks, changing their number count (f). eFLM — epifluorescence microscopy; LM — light microscopy; my — microns.

Step 1: Freeing Petrous Bone From Its Surroundings. In the autopsies, it was possible to take out the pertinent part of the temporal bone either with a fine-bladed handsaw after initial degloving of the scalp beyond the external auditory canal or with a Schuknecht oscillating trephine[31] (Stryker, Kalamazoo, Michigan) with subsequent freeing from the soft tissues of the skull base. Careful, nontraumatic sharp and blunt removal of soft tissue from the specimen followed.

Ideally, the handsaw and trephine method should be tested against bulk staining of temporal bones in situ. This has not been feasible, but the machine-drilled bone plug was compared with the traditional bulk tissue obtained by careful hand-sawing, to establish trephination as the routine method.

Step 2: Bulk Staining in Basic Fuchsin. The entire specimen was immersed in 1% basic fuchsin dissolved in ethanol. Increasing the percentage of ethanol during the staining has been recommended, and schedules for the procedure have been proposed.[28,32] With no known experience of the adequate duration of temporal bone immersion or of the effect of dehydration on microfissures with increasing ethanol percentage, it was decided to test a procedure as simplified as possible for multiple routine use in the laboratory. All bulk staining was therefore executed in covering amounts of 1% basic fuchsin (Certistain fuchsin, Merck, Darmstadt, Germany) in 62% (wt/wt) pure ethanol, enhanced by a partial vacuum (0.8 bar, 10 minutes).

The consequences of dehydration by the ethanol solvent were unknown. If some shrinkage took place, cracking of the dried, brittle capsular bone would be anticipated. It was therefore necessary to control the effect of the 62% ethanol solvent of the basic fuchsin stain. With a fluorescent dye (0.5% wt/vol, Epodye, Struers, Copenhagen, Denmark) easily soluble in monomer methyl methacrylate (MMA) and used within materials science to detect material microfissures, direct immersion of control specimens into the fluorescent monomer solution could serve as a control. The optimum duration of immersion in basic fuchsin had to be established. Because of the varying bone density of the specimens and the inevitably varying thickness of the slices, no objective means for evaluation of the penetration of the stain existed. Sufficient staining of a specimen was defined as definite basic fuchsin–violet in canaliculi and osteocyte lacunae, in vessels and nerves, and in the center of the tissue. The bone matrix itself should be unstained or only slightly pink. Unfavorable overstaining was evident in cases of puddles of stain in conjunction with basic fuchsin–stained structures or surfaces. The ordinary bulk staining process for temporal bones lasted for months. To advance the stain-perilymph exchange and thus the stain diffusion time, the effect of drill holes in the arcuate eminence, giving access to the superior semicircular canal, was examined. Similarly, the use of microwaves was tested. It is established that the heat generated by microwaves, arising within the tissue bulk, is more uniformly distributed than that caused by ordinary incubation, in which the heat is transferred from the surface to the middle of the tissue. Microwaves thus facilitate the heat-dependent diffusion of stain, and the processing times were expected to be shortened significantly.[33] We used a laboratory microwave oven with continuous mode as an option and user-defined temperature constancy (45°C), obtained by short-pulsed sensor feedback magnetron inhibition, 40% effect (PP-780, Ax-Lab, Copenhagen).

Step 3: Procedures for Removing Surplus Stain and Remaining Fat and Water. The bulk-stained specimen was transferred from the stain and kept in the first, 93% (wt/wt), ethanol concentration for 1 day and then in 99% (wt/wt) ethanol for 1 day; then put in an ether-acetone 1:1 solution (vol/vol) twice within 3 to 4 days for defatting; then exchanged in 99% (wt/wt) ethanol twice, once a day. All liquid exchanges were followed by at least 10 minutes of use of a partial vacuum (0.8 bar, except for ether-acetone, which boils at close to 0.5 bar).

Step 4: Plastic Embedding. We used monomer MMA (BDH, Poole, England) in the whole series. It is stabilized by the addition of 0.01% hydroquinone, and usually this is removed by laborious washings and the monomer MMA is prepared for polymerization by short heating and addition of a catalyst, most frequently benzoyl peroxide.[34]

Some early specimens were handled in this usual way, but for routine use, the procedure was demanding, and it was decided to elaborate on the shortcut proposed by Buijs and Dogterom[35]: the hydroquinone was not removed, but its stabilization was surmounted by use of larger amounts of catalyst, and the benzoyl peroxide was exchanged with bis(4-tert-butylcyclohexyl)peroxydicarbonate (BBPD; Perkadox 16, Akzo, Deventer, the Netherlands). No heating of the monomer MMA solution was done. The following procedures were undertaken: 1) infiltration with monomer MMA, no catalyst, once, 1 day or more; 2) embedding with monomer MMA, 0.2% BBPD, once, 1 day; and 3) embedding with monomer MMA, 0.5% BBPD, once, until polymerization was achieved, duration highly variable (days to months).

Step 5: Cutting. All plastic-embedded specimens were cut with Bakelite cutting wheels (125 mm in diameter, 500 µm thick) on a precision cutting/milling machine (Accutom-2, Struers), with the specimen holder advanced 800 µm per slice, producing a mean of 17 slices, 250 to 300 µm thick, per otic capsule.

To test this step, we performed experiments with ultrathin diamond-coated cutting wheels (125 µm thick; Struers) in order to minimize the tissue loss in the cutting process.

Step 6: Grinding. The machine-cut slices ("raw" slices) were fixed on a plastic slide with double-adhering Scotch tape, wet hand-ground by coarse and fine sandpaper to about 150 µm ("coarse" slices), removed from the tape by hexane, and remounted on the plastic slide with cyanoacrylate glue (Scotch-Weld, 3M, Zurich, Switzerland). Some of the slices were further hand-ground to 30 to 50 µm ("fine" slices) and mounted with cyanoacrylate glue.

Step 7: Analysis. "Fine" slices could be analyzed by light microscopy or epifluorescence microscopy; all other thicknesses could only be analyzed with the latter.[36] All microscopic examinations were performed blindly, without any information related to the individual. For statistical reasons, only unilateral analyses were performed. The primary test parameter was the number of capsular microfissures of either the Mayer type or the Frost type. Mayer-type microfissures were defined as the rather short stained microfractures radiating from or crossing the perilymphatic spaces, as first described by Mayer.[1,2] The larger, often communicating microfissures, as also described by Mayer, extending from the vestibule to the facial canal or from the posterior ampulla to the

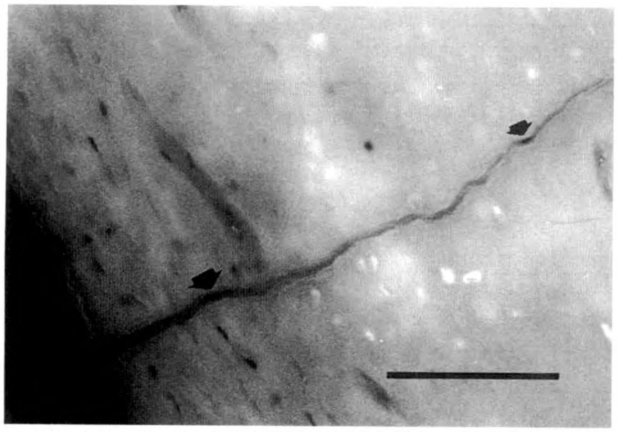


Fig 3. Well-defined Mayer-type microfissure extending from perilymphatic space with visible intraluminal cells (arrows) and more diffusely stained connective tissue (epifluorescence microscopy, original ×400). Note that microfissure is narrower than nearby capillaries. Bar — 100 μm.

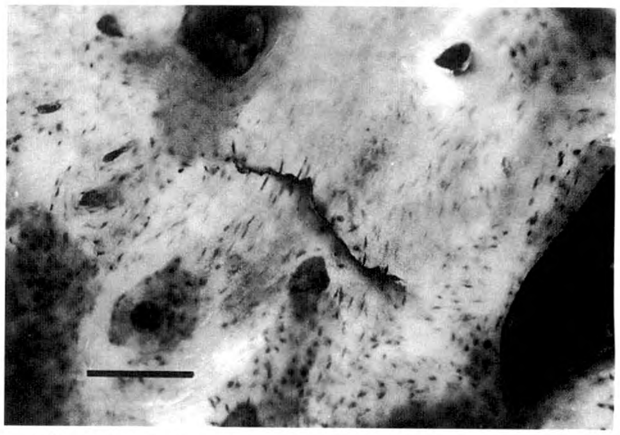


Fig 4. Well-defined, large Frost-type microfissure in bone matrix, without relation to luminal structures such as vessels or marrow spaces (epifluorescence microscopy, original ×100). Microfissure exhibits good stain uptake and visible crack at surface level, but no luminal contents. Bar — 250 μm.

round window niche, will be dealt with later and are not included in the present validations. Frost-type microfissures were defined as slender, often filiform, stained cracks in the bone substance, opening to the surface of inspection, as described by Frost.[28] Mayer-type microfissures apparent within the heavily stained surface projections of the endothelial lining of the membranous labyrinth, as well as the Frost-type microfissures seen within the projections of the walls of heavily stained structures such as vessels and nerves, were only included if they presented outside the lining of the structure. Penetration throughout the thickness of the histologic slice of a microfissure of either type as initially required by Frost was not found to be justified, because of the thick slices. Doubtfully stained microcracks were registered as such.

Counting was performed with the the CAST-Grid system (Olympus, Copenhagen) attached to a BX50 Olympus microscope (Olympus, Tokyo, Japan). The CAST-Grid system is a semiautomatic computer-assisted device for microscopic stereological estimation. Any area of interest may be outlined on-screen by the cursor at low-power magnification. Then, this area can be scanned at higher magnifications by computer-controlled movements of the motorized object table, in the so-called meander pattern, covering the whole delineated area.[27] This function ensured that any structure was seen once and only once. The detection of microdamage was undertaken at optic magnification ×40 with the meander sampling function, and when a crack of either type was identified, we confirmed and measured it at ×100 or at ×400. Epifluorescence microscopy was performed with a 100-W mercury burner (Osram, Berlin, Germany) and a tetracycline exciter cube (400 to 440 nm).

Statistics. The effects of the various procedures were tested with double-sided, independent t-tests, and the grinding effect was tested with paired t-tests. The effects of the duration of immersion in basic fuchsin and of the duration in the microwave oven were tested by analysis of variance.

RESULTS

Using any modification of these steps, we have been able to demonstrate well-stained, well-defined microcracks in the compact bony otic capsule of the Mayer type (Fig 3), as well as the Frost type (Fig 4).

Step 1. No statistically significant differences in the number of Mayer-type or Frost-type microfissures were shown for the exclusively hand-sawn adult otic capsules as compared to the trephined ones (p > .28; see Table).

Step 2. When bulk-stained with Epodye, larger

NUMBER OF MICROFISSURES FOR VARIOUS ALTERNATIVE PROCEDURES USED BEFORE STAINING (N = 136)

	Microwave Oven			*Freeing of Temporal Bone*			*Drilling of Arcuate Eminence*		
Microfissures	*No (n = 37)*	*Yes (n = 99)*	*p*	*Trephine (n = 123)*	*Handsaw (n = 13)*	*p*	*No (n = 42)*	*Yes (n = 94)*	*p*
Mayer-type	15.2	16.4	.58	16.2	15.1	.75	15.0	16.6	.46
Frost-type	3.8	6.6	.02*	5.6	8.1	.27	5.4	6.1	.64

Because of evidence of influence of microwaves, tests for freeing specimens and for drilling were made separately for subgroups with and without microwave exposure, and no differences were found (not shown).

*Statistical significance at 5% level.

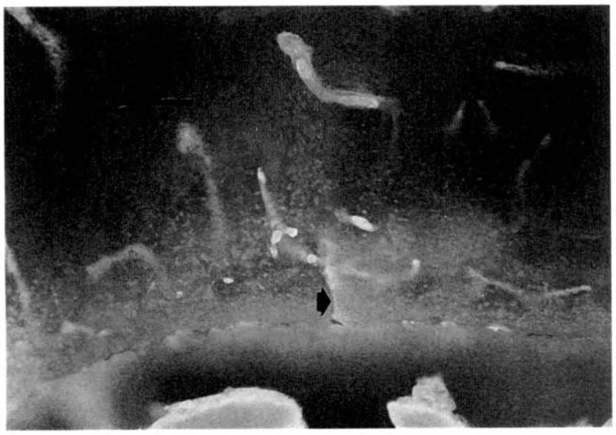


Fig 5. Specimen immersed in monomer methyl methacrylate with fluorescent dye (epifluorescence microscopy, original ×40). Mayer-type microfissure (arrow) is visualized by dye, extending from posterior semicircular canal. In empty lumen, smaller spots of dyed methyl methacrylate are seen. Bar — 500 µm.

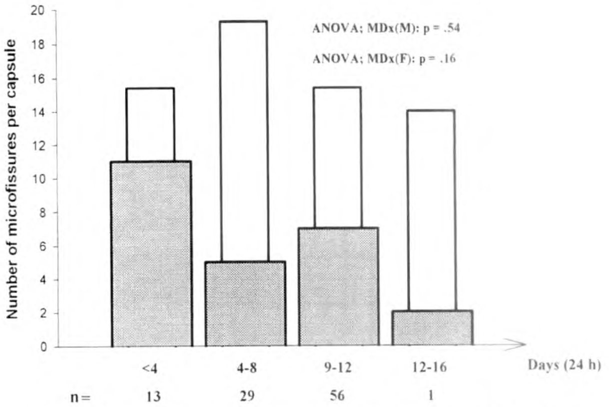


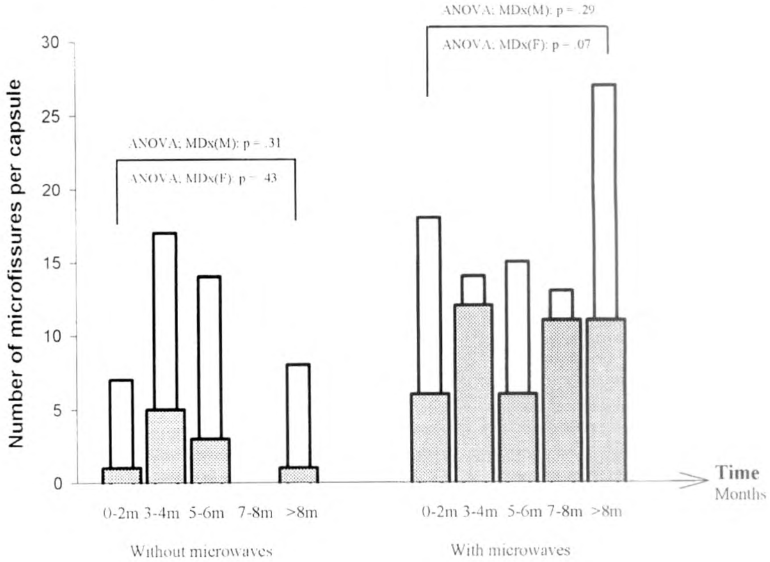
Fig 7. Number of capsular microfissures related to length of microwave exposure, divided into 4-day groups. Slender bars represent MDx(M), and broad bars MDx(F). No sign of linear relationship between crack number and duration is seen, and groups do not differ significantly (4-group ANOVA, p > .16).

Mayer-type microfissures with fluorescent content were found next to the perilymphatic spaces (Fig 5), whereas smaller microfissures, especially Frost-type microfissures, could only occasionally be identified convincingly. For adult human temporal bones, an immersion time between 2 and 3 months gave sufficient staining throughout the bone. Immersion of less than 1 month risked understaining, whereas no conflicting overstaining was seen in the few specimens left for 4 months or more. Drill holes did not affect the surrounding bone and did not augment the microfissure number ($p \geq .5$; see Table). Use of microwaves visibly advanced the staining, whether used intermittently or continuously, and a convenient schedule for routine laboratory use was 45°C, 8 hours daily, for 8 to 12 days. No linear influence of the duration of staining or of the duration of microwave exposure on the number of microfissures was demonstrated (Figs 6 and 7), but the final analysis showed an either-or effect of microwave exposure of any length as compared to none on the number of Frost-type microfissures (*t*-test, $p = .02$; see Table and Fig 8). No such influence was seen on the number of Mayer-type microfissures ($p = .58$; see Table).

Step 4. We found BBPD to be easier to control than benzoyl peroxide. The polymerization passed on satisfactorily, and only a few CO_2 bubbles were captured in the polymerized MMA. Thus, the effect of the hydroquinone was safely overcome. The penetration of the monomer MMA was good, particularly for the specimens with drilled access to the labyrinth. However, plastic embedding was never fully predictable, and some specimens had to have the monomer MMA with 0.5% BBPD renewed because of the absence of polymerization.

Step 5. Because of the large size of the specimens

Fig 6. Number of capsular microfissures related to length of bulk staining in basic fuchsin, subdivided into 2 groups with regard to microwave exposure. Slender bars represent Mayer-type microfissures (MDx(M)), and broad bars represent Frost-type microfissures (MDx(F)). No sign of linear relationship between crack number and duration is seen, and 2-month groups do not differ significantly within subdivisions (5-group ANOVA, p > .07).



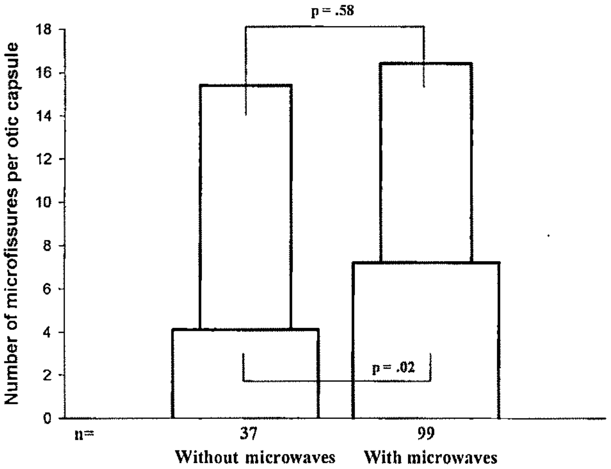


Fig 8. Number of capsular microfissures related categorically to no or any microwave exposure. Slender bars represent MDx(M), and broad bars MDx(F). Treatment of bulk specimens with microwaves of any duration is seen to augment number of MDx(F) significantly (p = .01). No effect of microwaves is seen on MDx(M).

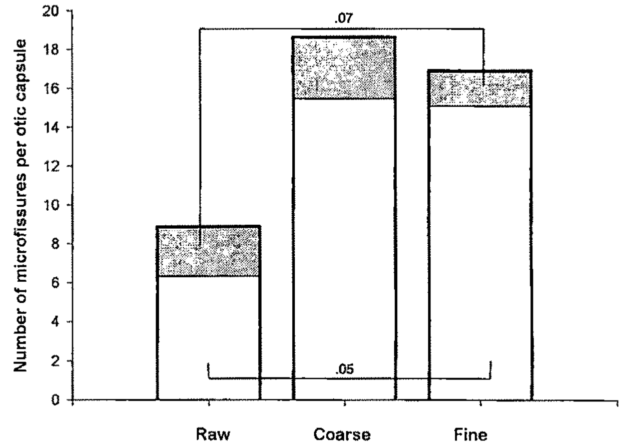


Fig 10. Number of bulk-stained MDx(F) in paired series of 8 human otic capsules, treated with microwave exposure and analyzed successively at all 3 grinding levels: raw, coarse, and fine (*t*-tests, p values shown for various "raw-fine" comparisons; *t*-test p values attached to shaded areas represent test values for all microfissures of that type, ie, both accepted and doubtful microfissures).

(40 mm in diameter) and the wobbling of the periphery of the thin diamond wheel, many slices were lost unpredictably when the specimen holder was advanced 600 to 700 μm per slice, and the use of such cutting wheels was abandoned early.

Step 6. Unpaired comparisons of specimens ground to "raw," "coarse," and "fine" levels revealed an increased number of Frost-type microfissures with an increased level of grinding in the microwave-treated group (p < .0005; Fig 9). Supplementary paired comparisons of Frost-type microfissures were made consecutively with microwave-treated specimens at all 3 levels (n = 8), showing the increase in number to happen from "raw" to "coarse" (Fig 10). No statistically significant increase was seen in the paired group without microwave exposure (n = 7). The mean number of Frost-type microfissures in the unpaired "coarse" microwave-treated series (6.74) differs significantly from that of the matched group of paired specimens (15.5; p = .006).

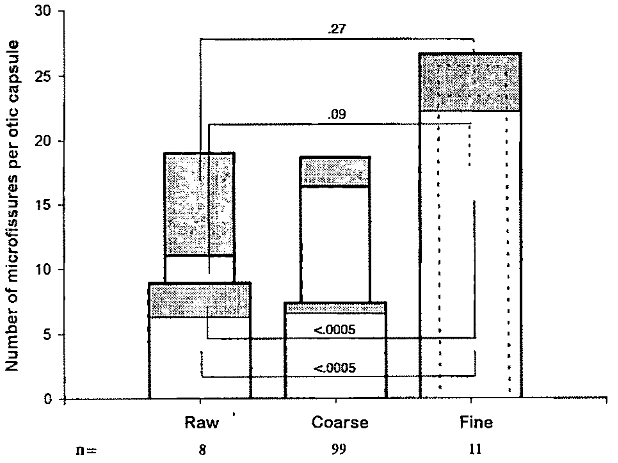


Fig 9. Number of bulk-stained microfissures in unpaired, independent groups of microwave-treated human otic capsules, analyzed at 3 different levels of grinding. Slender bars represent MDx(M), and broad bars MDx(F). Shaded areas represent doubtful microfissures of either type. Dotted lines represent MDx(M) bar, hidden by broader MDx(F) bar. (ANOVA p values attached to shaded areas represent test values for all microfissures of that type, ie, both accepted and doubtful microfissures.)

DISCUSSION

With all of the variations of the technique outlined above, we have been able to certify the existence of the Mayer and Frost types of microfissures in the human otic capsule in the neighborhood of the perilymphatic spaces. The presence of convincing microfissures in the Epodye specimens, identified before the bulk staining procedure, indicated they were not due to shrinkage provoked by dehydration during the immersion in basic fuchsin–ethanol, thus substantiating their intravital origin. In Epodye specimens, Mayer-type microfissures were readily seen in overview magnifications (×40), whereas Frost-type microfissures were only sporadically registered because of the vague contrast to the bright fluorescent bone background, and they were not always definitively discernible from artifacts. Microdamage is traditionally identified in light microscopy mode. Recently, it has been validated that using the autofluorescence of basic fuchsin for analyses in fluorescent light mode gives results comparable to those obtained under the light microscope,[36,37] but using the epifluorescent light mode allows for gross measurements and counting, independent of the thickness of the slices. This allows the use of thick slices, as in the present series, and thus enables us to access the specimens early in processing, providing the possibility of testing these early procedures and simultaneously giving an advantage in spared laboratory work.

Identifying a crack turns out to be largely a matter of pattern recognition. Most microfissures are primarily recognized as a disruption of the neighboring regular histologic surface and may in most instances be seen at low optic magnifications (ie, ×40). Larger intravital microfissures may be distinguished from processing cracks by a more smooth and regular course as compared to the rather violent zigzag course of the artifacts.

Nevertheless, the mere pattern of discontinuity will not suffice for separating the cracks. Smaller cracks cannot be differentiated by their appearance at all. With prestaining, the separation of intravital from artifactual cracks may be obtained in most cases for both larger and smaller cracks.

Still, some major problems of differentiation arise. First, a crack may produce an illusion of good staining, if it is situated in a projection of the wall of a heavily stained structure, such as the walls of the perilymphatic spaces, air cells, nerves, or vessels. We have therefore discarded all cracks found entirely inside other stained structures, however good the stain may appear. Second, a prominent problem was differentiating larger Mayer-type microfissures with vascular ingrowth from artifacts along a vessel. However, such artifacts may not present staining of the entire lumen and, in particular, may be discriminated from genuine in vivo fissures by scrolling the focus up and down; an artifact will only show good staining at the height of the limited extension of the vessel, whereas an intravital fissure should be prestained all through its depth. Further, a vessel may have a surrounding osteonal structure, while a crack with ingrowth of a vessel does not. Usually, a combination of pattern recognition and staining will suffice at low magnification to classify the cracks as intravital or artifactual, leaving doubtful cases to be registered as such. Third, in areas in which the bone matrix has received a rose background staining, the vague color of the walls may provide the smaller artifacts with the erroneous look of a faintly stained true intravital microfissure. However, genuine Frost-type microdamage is very sharply demarcated by its bright violet staining. Recently, use of double epifluorescence microscopy analyses at 2 different wavelengths has been found advantageous in discriminating intravital microfissures from artifacts.[37]

Pioneers within the field of bulk staining[28,30,32] have recommended specific schedules for the processing of long bones. Our goal has been to develop a procedure with as few steps as possible without loss of information. Very dense and large bones, such as the petrous bone, were not expected to benefit from a direct application of all recommended steps. Any step in the processing was regarded as potentially producing artifacts, and any artifact arising before or during staining would possibly result in stained artifacts, inseparable from intravital cracks. For routine purposes, freeing the temporal bone with the trephine saved much time, because the cylindrical bone plugs were sized for the glass vials used in the later procedures. Thus, it was most convenient that the use of a trephine apparently did not result in microfissures, at least not more than the standard use of a handsaw. Drilling of the arcuate eminence to access the superior semicircular canal was a way to obtain good penetration of the basic fuchsin, as well as the MMA, to the perilymphatic spaces in order to provide good overall staining and embedding with as much support to the fragile structures as possible. The drilling did not result in more frequent microfractures, either locally or globally (see Table). Thus, the major disadvantage of this otherwise beneficial procedure is the focal elimination of existing cracks in the drill hole.

A major concern has also been whether increasing alcohol concentrations in the basic fuchsin solution would inflict an adverse dehydration on the perilabyrinthine bone with ensuing artifactual cracking, since such cracking would pick up stain, imitating a genuine intravital microfissure. Thus, we did not change the initial 62% (wt/wt) pure ethanol solvent with increasing percentages of ethanol as recommended. We have not noticed any adverse effect of this restricted imbibition so far, but of course, a missing stain will not be recognized. Specimens accidentally processed at too-high temperatures, after inadvertent total evaporation of stain, showed serious signs of overstaining and debonding of the bone matrix structure: puddles of basic fuchsin, and multiple stained streaks in the matrix without convincing physical discontinuity. The statistically significant influence of well-controlled microwaves on Frost-type microfissures (see Table and Fig 8) may represent the desired enhancement of thorough staining of the tissue bulk, with efficient staining of all (or at least more) of the smaller intravital cracks that otherwise might be missed or discarded as artifacts. The increase in Frost-type microfissures could be suspected to be artifactual, but this idea is countered by the simultaneous general enhancement of tissue staining with time and by the lack of time-dependence of the prestaining of Frost-type microfissures (Fig 7). Further, the continued absence of microfissures after microwave treatment for those specimens usually without microfissures, ie, animals and infants (to be discussed in a later publication), speaks against an artifactual increase. That Mayer-type microfissures are unaffected by the better stain penetration may be explained by their direct extension from the stain-imbibing perilymphatic spaces. Thus, at present, counts of Mayer-type microfissures are considered to be independent of microwave exposure, while the Frost-type microfissure counts without microwave enhancement of the stain infiltration only reveal half of the present intravital Frost-type cracks.

The groups of "raw" and "fine" specimens are small, as is the series of consecutive grinding, with very high variations in crack number within each group. Demonstration of higher Frost-type microfissure counts for "fine" specimens in the unpaired microwave-treated series (Fig 9) could only partially be ascertained by paired analysis for the microwave-treated controls with consecutive grinding (Fig 10). This apparent "grinding effect" may be a matter of type I error, by primarily being group-dependent. This idea is supported by the significant difference in mean number of Frost-type microfissures between the unpaired and paired series of "coarse" specimens (6.7 versus 15.5; p = .006). However, in all the comparisons, there is a tendency for more Frost-type cracks to be observed after grinding, and the paired series suggests that this increase takes place from "raw" to "coarse," and not from "coarse" to "fine." This may be a consequence of the thinning of the slices, which may obscure the continuity of tortuous cracks at the surface level, giving rise to repeated counting of the same partitioned crack (Fig 2), but a reduction of surface projections of stained structures that hide minor cracks may truly elevate the counts. Further, histologic details may be presented better in thinner slices, because of less blurring of the fluorescence. This is supported by the fact that the entire increase in the microfissure count is found in the small Frost-type microfissures, while the amount of larger Mayer-type microfissures, which could be suspected to increase during the tissue handling as well, is constant.

Having identified cogent intravital microfissures with our present staining schedule opens the way for more advanced staining procedures. Therefore, we can recommend our simple procedure for the temporal bone, but do not discard a possible role here for the more stepwise staining advocated for long bones. We recommend use of microwaves during staining in studies on smaller microdamage. Large and small microdamage may be investigated reliably at "coarse" or finer levels of grinding. The "coarse" level of grinding seems an acceptable compromise between scientific demands and laboratory efficiency.

Thus, the presented technique is suitable for further research on the role of microfissures in the etiology and pathogenesis of otosclerosis, but it is also anticipated to be a valuable tool in future basic research on perilymphatic leakage in which large pathological cracks are communicating with the middle ear.

ACKNOWLEDGMENTS — We thank Bo Hansen, Pathology Department, Rigshospitalet, for his skillful assistance in procuring the biopsy specimens, and Neste Kemi A/S, Hørsholm, Denmark, for providing the Perkadox 16. We thank Ingeborg Olsen and Sanne Friisnæs for their continued and devoted efforts in the processing of the specimens.

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Ann Otol Rhinol Laryngol 110:2001

PHOTOCHEMICALLY INDUCED EQUILIBRIUM DYSFUNCTION IN THE HAMSTER MODEL WITH EVALUATION BY MEANS OF A NEW GLOBE ROTATORY TEST SYSTEM

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Thrombosis in the inner ear is regarded as one of the causes of equilibrium dysfunction. We have established an experimental thrombosis model by producing a photochemical reaction between rose bengal and green light, and have evaluated the dysfunction with a new rotatory test system. Hamsters were treated with tissue-type plasminogen activator (tPA) in doses of 0, 0.13, 0.26, and 0.52 mg/kg. The equilibrium dysfunction of the hamsters was evaluated by scoring their behavior according to visual observation and by measuring their time on the rotatory test system. Treatment of animals with sufficient tPA (≥0.26 mg/kg) caused a significant amelioration of the behavior and a concomitant significant prolongation of time on the rotating globe. These findings suggest that the equilibrium dysfunction induced by the photochemical reaction was due to the thrombi formed, and that our test system may provide a useful tool for evaluating equilibrium dysfunction in hamsters.

KEY WORDS — equilibrium dysfunction, hamster, human tissue-type plasminogen activator, inner ear, photochemically induced thrombus.

INTRODUCTION

Sudden-onset vertigo with subsequent equilibrium dysfunction is thought to indicate a microcirculatory disorder in the inner ear.[1-4] Several animal models of circulatory disorders in the inner ear have been described. Igarashi et al[5] produced arterial emboli in the inner ear microcirculation by intra-arterial injection of polystyrene microspheres. More recently, simple and reproducible experimental thrombosis models have been established that use the photochemical reaction between a systemically injected photosensitive dye, rose bengal, and transilluminated green light (wavelength, 540 nm).[6-9] The photoexcitation of rose bengal by green light yields singlet molecular oxygen within the vasculature by energy transfer. Intraluminally generated singlet oxygen and free radicals cause endothelial injuries followed by localized platelet adhesion, aggregation, and, finally, formation of a platelet- and fibrin-rich thrombus.[10-13] In the present study, we have applied this technique to the hamster inner ear and tried to quantitatively evaluate the behavioral changes due to the equilibrium dysfunction subsequent to photochemical intervention in the inner ear. The hamster is such a small and tame animal that it is easily handled, and it has a short tail that cannot be used for maintenance of its posture. For these reasons, we chose the hamster as an experimental equilibrium dysfunction model.

Recombinant human tissue-type plasminogen activator (tPA) is a thrombolytic therapeutic agent, the efficacy of which has been well documented, not only in several animal models, but also in clinical studies. For example, a single intravenous bolus injection of tPA significantly decreased the ischemic area in middle cerebral arterial thrombosis in a rat model,[6,14] and treatment with intravenous tPA within 3 hours of the onset of ischemic stroke improved clinical outcome at 3 months.[15]

In the present study, we established a hamster model of equilibrium dysfunction by formation of thrombi in the inner ear microcirculation, and evaluated the resulting equilibrium dysfunction by quantitatively estimating the behavioral changes in the hamster with the use of our new rotatory test system. The use of tPA in reversing the dysfunction confirmed the formation of thrombi in the inner ear.

MATERIALS AND METHODS

Animals. Twenty-eight (4 animals for morphological study) male hamsters of the golden strain (SLC, Hamamatsu, Japan) weighing 100 to 130 g were used throughout the study and fed a standard chow (MF, Oriental Yeast Co Ltd, Tokyo, Japan). All hamsters were confirmed to have a normal Preyer's reflex before enrollment in the experiments. All experiments were performed in accordance with our institutional

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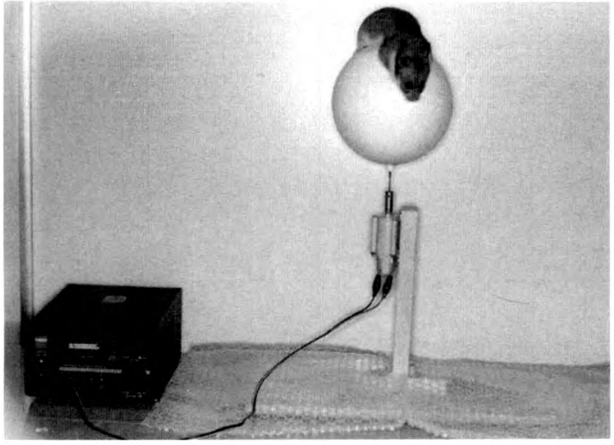

Fig 1. New globe rotatory test system device. Globe made of styrene foam with diameter of 12 cm was connected to DC electric motor. Hamsters were placed on top surface of globe, and globe began to rotate at 36 rpm. Time required for animal to fall from surface of globe was measured.

guidelines for animal studies.

Agents. The tPA was kindly supplied by Toyobo Co, Ltd (Osaka, Japan). The rose bengal was obtained from Wako (Osaka). The other chemical substances were obtained from Sigma (St Louis, Missouri).

Induction of Thrombosis in Inner Ear. On day 0, the animals were ventilated with 1% halothane while on a respirator (Harvard 683, Natick, Massachusetts). Their body temperature was maintained at 37°C with a heating pad (Gaymer, Valencia, California). A catheter (inside diameter, 0.4 mm; outside diameter, 0.8 mm; Natsume, Tokyo) was inserted into the right jugular vein for the injection of rose bengal solution or tPA. The left middle ear was exposed by a postauricular approach, and the middle ear was exposed. Under an operating microscope, part of the temporal bone was drilled away to expose the epitympanum. The tympanic membrane, malleus, and incus were carefully removed, with the inner ear left uninjured. A photochemical reaction was initiated by systemic injection of rose bengal combined with green light transillumination. Rose bengal (20 mg/kg) dissolved in physiological saline solution was injected intravenously via the right jugular vein at the rate of 1.0 mL/kg as a single bolus. Photoillumination of the vestibule through the oval window was started 5 minutes after the injection of rose bengal, with a 3-mm-diameter optic fiber (Hamamatsu Photonics, Hamamatsu) connected to a xenon light (wavelength, 540 nm; Hamamatsu Photonics). The head of the optic fiber, mounted on a fine manipulator, was placed 2 mm away from the oval window. During the experiment, the blood flow in the inner ear was measured by a laser Doppler flowmeter[16] (BRL-100, Bioresearch Center, Nagoya, Japan). A probe (BN-0.5, Bioresearch Center) was attached to a micromanipulator and fixed at a distance of 10 mm from the lateral bony wall of the vestibule. The inner ear blood flow was continuously monitored from 20 minutes before the initiation of transillumination until 60 minutes after the initiation. Finally, the catheter was removed, the wounds were closed, and the hamster was allowed to recover from anesthesia.

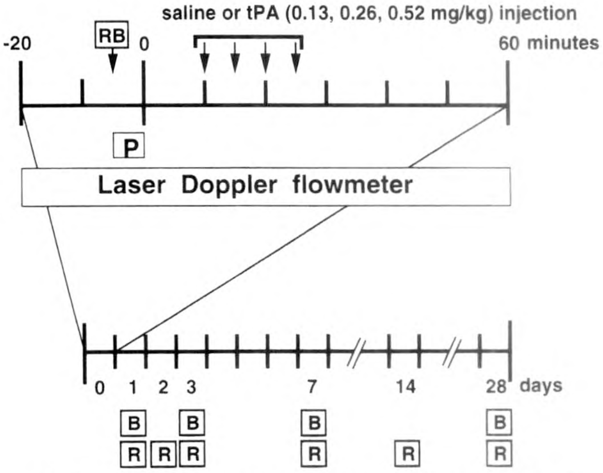

Fig 2. Experimental protocol. After rose bengal (RB; 20 mg/kg) injection, photoillumination (P) of vestibular organ through oval window was done for 5 minutes. Saline solution or tissue-type plasminogen activator (tPA) was injected in 4 equally divided bolus doses at 5-minute intervals. Control animals were given saline solution. After operation, we performed evaluation of behavioral changes by visual observation (B) and globe rotatory test (R).

Evaluation of Behavioral Changes by Visual Observation. At various intervals after the completion of photoillumination, the hamsters were tested for integrity of vestibular function. This was done by ob-

TABLE 1. CHANGES IN INNER EAR BLOOD FLOW MEASURED WITH LASER DOPPLER FLOWMETER

	Time (min)				
	–15	*15*	*30*	*45*	*60*
Control (n = 6)	52.5 ± 8.6	17.8 ± 4.4	16.8 ± 5.1	13.7 ± 4.9	13.0 ± 2.0
tPA 0.13 mg/kg (n = 6)	57.0 ± 5.0	14.4 ± 6.5	8.0 ± 2.4	11.3 ± 4.2	12.2 ± 4.9
tPA 0.26 mg/kg (n = 6)	40.7 ± 4.2	24.4 ± 7.3	22.7 ± 7.2	32.3 ± 7.1*	27.3 ± 6.8
tPA 0.52 mg/kg (n = 6)	52.7 ± 8.5	24.5 ± 4.3	35.8 ± 7.0*	41.9 ± 9.0*	40.3 ± 8.7*

Changes in inner ear blood flow associated with thrombolysis by tissue-type plasminogen activator (tPA) are summarized. Data are mean ± SEM (blood flow in millivolts).

*p < .05 versus control.

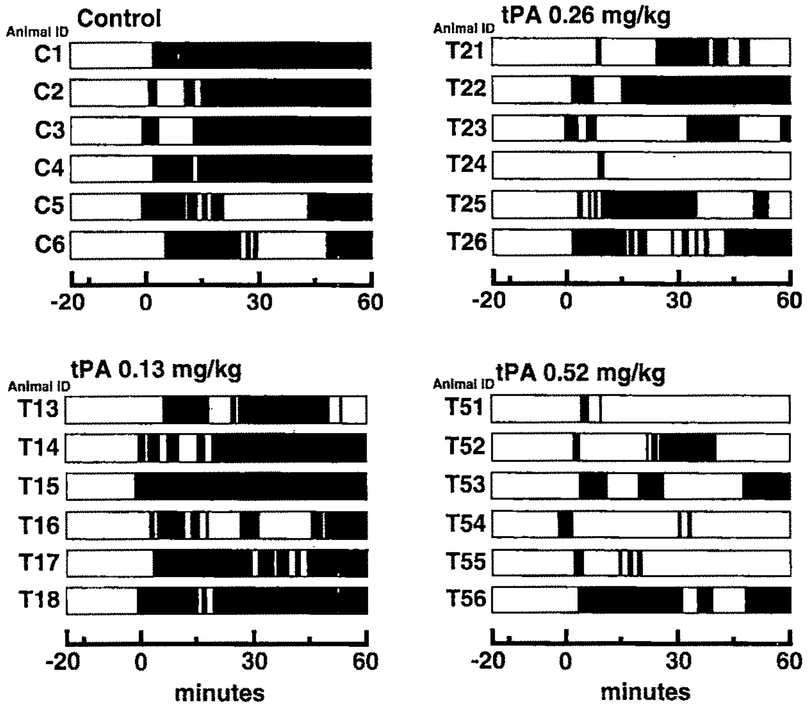

Fig 3. Time profiles of inner ear blood flow measured by laser Doppler flowmeter are given schematically. Solid black bar represents period of time in which blood flow was decreased by 70% or more as compared with baseline value measured before administration of RB. Time scale is same as in Fig 2. Inner ear blood flow was monitored from 20 minutes before initiation of photoillumination until 60 minutes after initiation.

serving the behavior of the hamsters on a flat floor. The animal's behavior was monitored for several minutes by the same observer throughout the series of experiments and graded as follows: grade 1, normal posture and walking; grade 2, behavior with inclined head; grade 3, behavior with horizontal rotating; grade 4, behavior with rotating along the longitudinal axis. Observations of these behavioral changes in hamsters on the flat floor were made on days 1, 3, 7, and 28.

Evaluation of Equilibrium Dysfunction by Globe Rotatory Test. The device used for evaluation of equilibrium dysfunction was a new globe rotatory test system, consisting of a rotating globe connected to a DC electric motor (Tamiya Co Ltd, Tokyo) and a stable stand for fixation of the globe and motor. The globe was made of styrene foam with a diameter of 12 cm (Fig 1). The rotation of the globe about the vertical axis was driven by a DC electric motor, with the rate adjusted to 36 rpm. The landing platform was covered with a soft plastic cushion placed 25 cm down from the top of the globe so that the animals would not be harmed if they fell from the surface of the globe. The hamsters were placed on the top surface of the globe, and then the globe was rotated at 36 rpm. The time during which the animals maintained their equilibrium on the rotating globe was recorded. The measurement was repeated 3 times at intervals of more than 3 minutes, and the mean was calculated on days 1, 2, 3, 7, 14, and 28. Since all normal hamsters tested in the preliminary trial could hold on to the surface for more than 60 seconds without falling, the duration of the experiment was set at 60 seconds. The time was counted as 60 seconds if the animals stayed on for a longer period.

Microscopic Observation. The animals were painlessly killed with an overdose of pentobarbital sodium. The temporal bones were surgically dissected, fixed with 10% formalin for 10 days, and then decalcified in 2.5% trichloroacetic acid for another 10 days. After fixation and decalcification, the specimens were washed in running water for 12 hours.

TABLE 2. BEHAVIORAL CHANGES SEEN BY VISUAL OBSERVATION ON FLAT FLOOR

	Day 1				*Day 3*				*Day 7*				*Day 28*			
	1	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*
Control	0	0	1	5	0	1	3	2	0	3	1	2	1	1	1	0
tPA 0.13 mg/kg	0	0	2	4	0	1	3	2	0	4	2	0	1	1	0	0
tPA 0.26 mg/kg	1	3	2	0*	5*	1	0	0	5*	0	0	0	4	0	0	0
tPA 0.52 mg/kg	2	2	2	0*	3	3	0	0	5*	1	0	0	4	2	0	0

Animal's behavior was monitored for several minutes by same observer throughout series of experiments and graded as follows: grade 1, normal posture and walking; grade 2, behavior with inclined head; grade 3, behavior with horizontal rotating; grade 4, behavior with rotating along longitudinal axis. Observations of these behavior changes in hamsters on flat floor were made on days 1, 3, 7, and 28.

*$p < .05$ versus control.

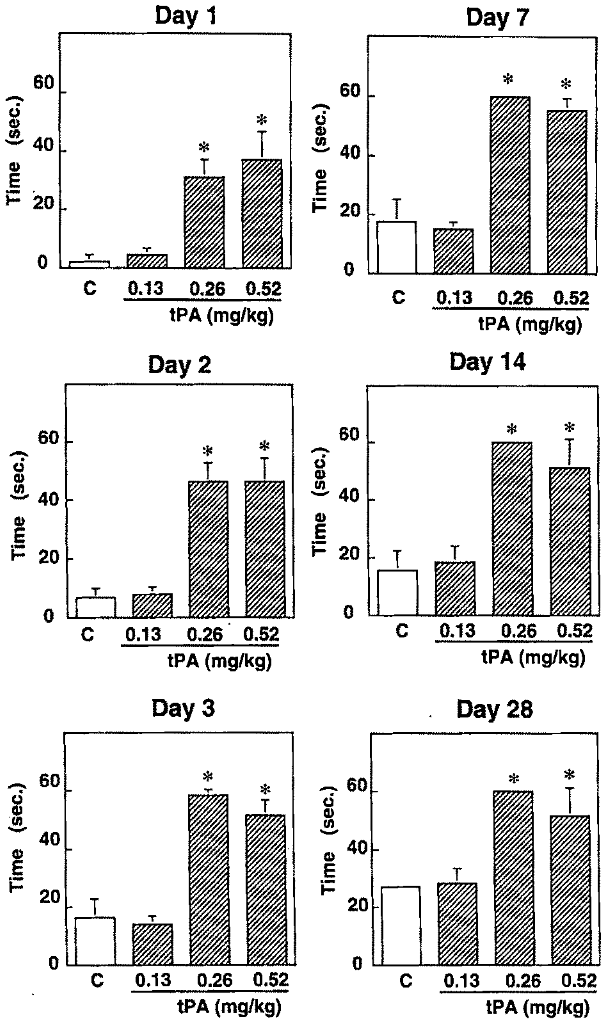

Fig 4. Globe rotatory test results on days 1, 2, 3, 7, 14, and 28. Open bars are measurements for control animals (C), and hatched bars are for animals treated with tPA. Time to falling from surface of globe was evaluated on days 1, 2, 3, 7, 14, and 28. Experiment was performed for 60 seconds. Time was recorded as 60 seconds if animal had not fallen within test period. * — $p < .05$ versus control animals.

After being neutralized in 5% anhydrous sodium sulfate overnight, the specimens were placed in distilled water for 12 hours. After dehydration in increasing concentrations of alcohol solutions (50%, 70%, 80%, 90%, and 100%), the specimens were embedded in increasing concentrations of celloidin (2%, 3%, 6%, and 10%) and sectioned. These sections were stained with hematoxylin and eosin and studied by light microscopy.

Electron Microscopic Observation. In order to further define a thrombus formation in the inner ear artery of the hamsters after photochemical reaction, we carried out electron microscopy. The animals were painlessly killed with an overdose of pentobarbital. The temporal bones were surgically dissected. These segments were prepared without rinsing to leave any formed platelets intact, and were fixed in 2.0% glutaraldehyde in 50-mmol/L sodium phosphate buffer for 30 minutes. Each segment was cut into several sections to allow visual inspection for scanning electron microscopy (SEM) as previously described.[6]

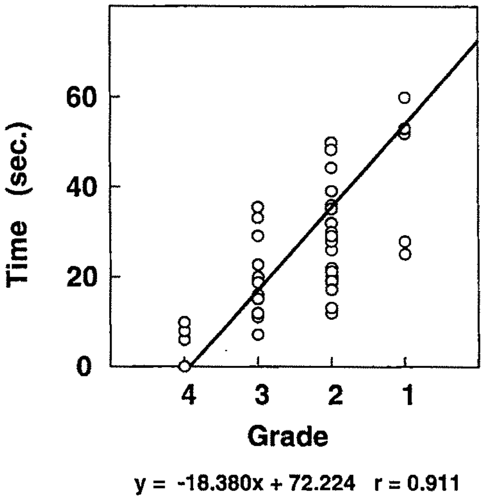

Fig 5. Correlations between results of behavioral inspection and globe rotatory test. Longitudinal axis is result of globe rotatory test (time), and horizontal axis is result of behavioral observation (grade; see text).

Experiment With tPA. The hamsters were divided into 4 groups (n = 6 each). The control animals were given saline solution after photoillumination. The other 3 groups were treated with tPA after photoillumination at doses of 0.13, 0.26, and 0.52 mg/kg, respectively. The time schedule of the tPA dosing was as follows. After transillumination with green light for 5 minutes, the thrombi were allowed to age for the next 10 minutes. Then, tPA was intravenously injected in 4 equally divided bolus doses at 5-minute intervals (Fig 2). The behavioral examination and rotatory tests were performed as described above.

Statistics. All data are expressed as mean ± SEM. The data were analyzed statistically with the χ^2 test or the Student-Newman-Keuls test.

RESULTS

Inner Ear Blood Flow. The changes that occurred in the inner ear blood flow are summarized in Table 1. The tPA significantly increased the inner ear blood flow at doses of 0.26 and 0.52 mg/kg after the initiation of thrombus by endothelial injury. It also prolonged the duration of inner ear blood flow (Fig 3). However, the lower dose of 0.13 mg/kg of tPA was much less effective, and the blood flow was almost the same as that of the controls. There was no significant difference in the baseline values of inner ear blood flow before the administration of drug among the 4 animal groups.

Effects of tPA Treatment on Behavioral Changes.

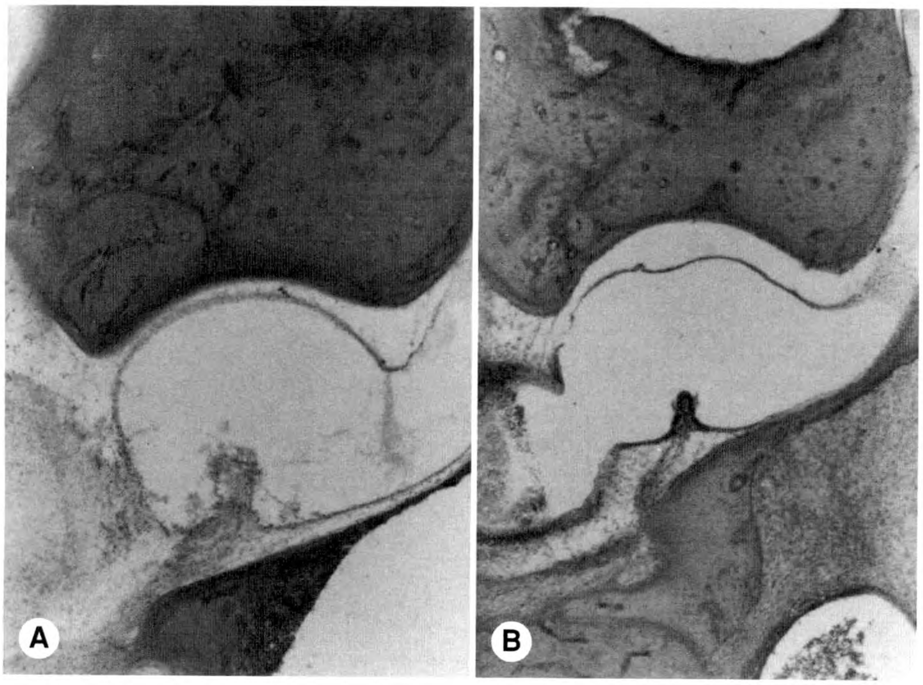

Fig 6. Light microscopic study performed 7 days after photoillumination (H & E, original ×100). **A)** Animal treated with RB, photoillumination, and saline solution injection. Hair cells in ampulla show disintegration. **B)** Animal treated with RB, photoillumination, and 0.52-mg/kg tPA injection.

The effects of tPA or saline solution on behaviorial changes seen by visual observation are shown in Table 2. The time the hamsters took to fall from the surface of the globe (generally, the hamsters fell while rolling down) was evaluated on days 1, 2, 3, 7, 14, and 28 (Fig 4). The time to falling in hamsters treated with tPA at doses of 0.26 or 0.52 mg/kg was significantly prolonged as compared with that of controls. Moreover, tPA enabled the hamsters to hold on to the surface of the rotating globe for a significantly longer period of time ($p < .05$) than control hamsters on all the days of observation. The correlation between the results of behavioral observation and the rotatory test was .911 (Fig 5).

Microscopic Observations. The morphological lesions of the control hamster were characterized by degeneration of the hair cells in the ampulla (Fig 6A). In contrast, degenerative changes were not observed and a normal appearance of the hair cells was maintained in the hamster treated with 0.52 mg/kg tPA (Fig 6B).

Electron Microscopic Observations. A densely packed thrombus formation was observed in the arteries of the inner ear in the control animal (Fig 7A). On the other hand, thrombus was not detected in the hamster treated with tPA at a dose of 0.52 mg/kg (Fig 7B).

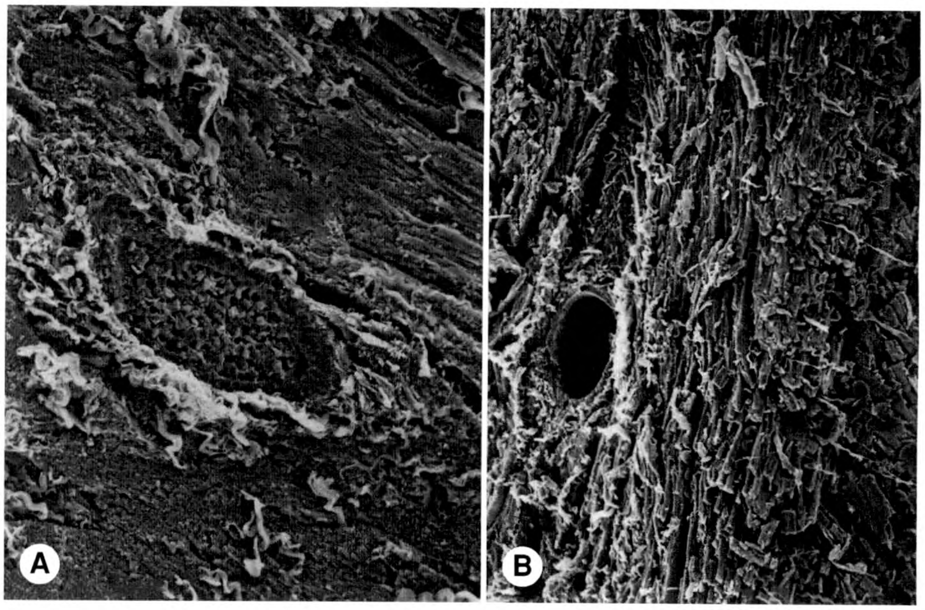

Fig 7. Scanning electron micrographs of inner ear arteries of hamsters (original ×900). These vessels were in proximity to ampulla of semicircular canals. **A)** Animal treated with RB, photoillumination, and saline solution injection. Photochemical reaction resulted in densely packed thrombus formation. **B)** Animal treated with RB, photoillumination, and 0.52 mg/kg tPA injection. Occlusive microthrombus was not detected in inner ear artery.

DISCUSSION

The aim of the present study was to develop a simple and reproducible model of equilibrium dysfunction induced by thrombotic occlusion in hamsters. In our morphological study, an extensive degeneration of the vestibular organs was observed 7 days after the completion of photoillumination in the presence of rose bengal (Fig 6A). These findings were similar to the ischemic lesions in association with arterial obstruction in the labyrinth reported by Kimura and Perlman.[3,4] The lesions produced were localized to only the illuminated area, since the cerebrum, cerebellum, and brain stem were not damaged by treatment with systemic injection of rose bengal combined with transillumination of the inner ear. Moreover, 0.52 mg/kg of tPA was enough to suppress the tissue damage produced by the photochemical intervention (Fig 6B). This finding suggests the dose of tPA that would lead to the sufficient lysis of clot fragments that had occluded the microcirculation of the inner ear. Thus, tPA allows the restoration of blood flow. The careful observation of behavioral changes and the evaluation of equilibrium dysfunction by our rotatory test supported the above finding, as treatments with tPA of 0.26 and 0.52 mg/kg significantly reversed the equilibrium dysfunction caused by the microcirculatory disorder. As a whole, the present findings indicate that the photochemical reaction caused thrombi only in the transilluminated site of the vestibular microcirculation, leading to circulatory occlusion sufficient to cause severe ischemic changes in our animals.

By using the photochemical reaction between photoillumination and rose bengal injection, we were able to establish a simple and reproducible model of equilibrium dysfunction in hamsters. Since our surgical and pharmacological interventions do not result in a direct injury to the bony labyrinth, bleeding or complicating infections were quite uncommon in our animals.

Tissue-type plasminogen activator dissolves intravascular clots and restores blood flow. However, the use of this agent for treating equilibrium dysfunction in humans may be limited by several possible complications, such as cerebral hemorrhage. In our experiments, however, 0.26 and 0.52 mg/kg of tPA decreased the severity of equilibrium dysfunction or even restored function. This result supports the conclusion that our equilibrium dysfunction hamster model operates by producing fibrin and platelet-rich clots.

For a more quantitative evaluation of the equilibrium dysfunction of the animals, we developed a new rotatory test device. We used the time (in seconds) to falling from the surface of a rotating globe as an indicator of equilibrium dysfunction and compared it to behavioral changes on a flat floor graded by visual observation (grades 1 through 4). There was close correlation between the results obtained with the rotatory test and the observation of behavior (Fig 5). However, the time required for the hamster to fall from the surface of the rotating globe was a more sensitive indicator of the effect of 0.26 mg/kg of tPA than were the graded behavioral changes.

It should be emphasized that the hamster is gentle and quite easy to handle and has a short tail. The long tail of an animal such as the rat might be an obstacle for estimating equilibrium dysfunction by the rotatory test, since it would be used for maintenance of posture. Taken together, the hamster model of equilibrium dysfunction and the newly developed rotatory test system may be very useful for evaluation of potential drugs for treatment of equilibrium dysfunction. Also, they could be used to evaluate the possible contributions of platelets and blood viscosity to the circulatory disturbance of the inner ear in normal and hyperlipidemic hamsters. Further study is, of course, needed to demonstrate the usefulness of the model and evaluation system.

In conclusion, we have established a relatively simple and reproducible hamster model of equilibrium dysfunction and a new rotatory test system that is quite suitable for quantitative evaluation of equilibrium dysfunction in hamsters.

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IMPORTANCE OF GLOTTIC CONFIGURATION IN THE DEVELOPMENT OF POSTERIOR LARYNGEAL GRANULOMA

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Posterior laryngeal granuloma is frequently related to 3 predisposing factors: vocal abuse, gastroesophageal reflux disease, and orotracheal intubation. It is strongly predominant in men and rare in women, except under postintubation circumstances, in which the incidence is higher in women. The aim of this study was to characterize laryngeal conformations for each sex that, whenever associated with different causes, may lead to the onset and particular location of granulomas, according to a main predisposing factor. Sixty-six subjects whose ages ranged from 18 to 73 years were studied. They were distributed into 4 groups according to the predominant cause of granuloma: intubation, vocal abuse, gastroesophageal reflux, and idiopathic causes. The larynx was evaluated during breathing, and the glottic proportion (GP) was measured. Glottic proportion is the mathematical ratio between the midsagittal dimension of the intermembranous region and that of the intercartilaginous region of the larynx during inhalation. Its measurement was feasible in 57 patients. The groups that had a causative factor other than laryngeal orotracheal intubation had GP values statistically similar to those of each other and to the control group of men, ie, close to 1.2. The postintubation group had GP values similar to the control group of women, ie, close to 1.0; this proportion protects the arytenoid region in women's larynges when there is effort during vocal production, but makes women susceptible to orotracheal postintubation granuloma.

KEY WORDS — granuloma, larynx, vocal fold.

INTRODUCTION

Posterior laryngeal granulomas occur frequently in the arytenoid region, normally over the vocal process, and are generally related to 1 of 3 predisposing factors: orotracheal intubation,[1-5] vocal abuse,[2-4,6,7] and gastroesophageal reflux disease (GERD).[3,4,8-10] In cases in which there is no evidence of the abovementioned factors, the granuloma is considered idiopathic.[3,4] Posterior granulomas are strongly predominant in men and rare in women, except when they occur after intubation, in which case the incidence is higher in women. Bloch et al[6] described 17 cases that were unrelated to previous intubation, Nasri et al[11] described 6, Öhman et al[8] 58, and Benjamin and Roche[7] 21, all of them in male patients. Feder and Michell[10] reported 1 case in a woman out of 22 patients studied.

Snow et al,[5] upon their study of 45 postintubation cases, found that 85% of the patients were female and pointed out the difference between the sexes in mucosa thickness of the vocal process of the arytenoid, which is approximately 59 μm in women and 97 μm in men.

In a descriptive case series, McFerran et al[3] reported 3 cases in male patients with GERD; 8 cases in patients with vocal abuse, including 1 woman; and 9 idiopathic cases, all of which were in men. However, when they analyzed postintubation granuloma, the incidence was much higher in women: 6 patients out of 9 were women (67%). The authors agreed with the hypothesis of Snow et al[5] that female larynges are smaller and have thinner mucosa and, consequently, are more susceptible to intubation trauma. They also related the male predominance in the remaining cases to stress and tension applied over the arytenoids, due to use of low frequencies and a hard glottal attack. According to the authors, the absence of women in the idiopathic group would suggest that there is something inherent in male larynges that would predispose them to a chronic inflammatory condition and formation of granuloma.

In 1983, Öhman et al[8] had already considered the hypothesis that constitutional factors would have an important role in the onset of the granuloma.

Searching for anatomic and morphological factors that would account for the differences observed in the incidence of granuloma in the sexes, we intended to characterize, according to the main predisposing factor, laryngeal conformations for each sex that, whenever associated with different causes, would lead to the onset of granulomas.

MATERIAL AND METHOD

We carried out a retrospective study of 66 cases of posterior laryngeal granuloma seen at the Instituto

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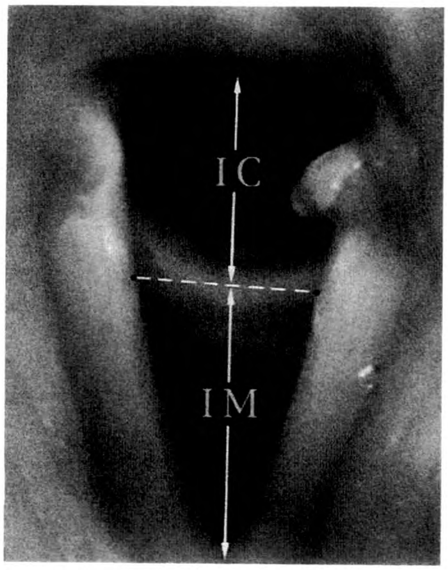

Fig 1. Image of glottic region shows intercartilaginous (IC) and intermembranous (IM) regions, with their respective midsagittal dimensions (arrows), whose limit is imaginary line (broken line) that crosses tips of vocal processes (points).

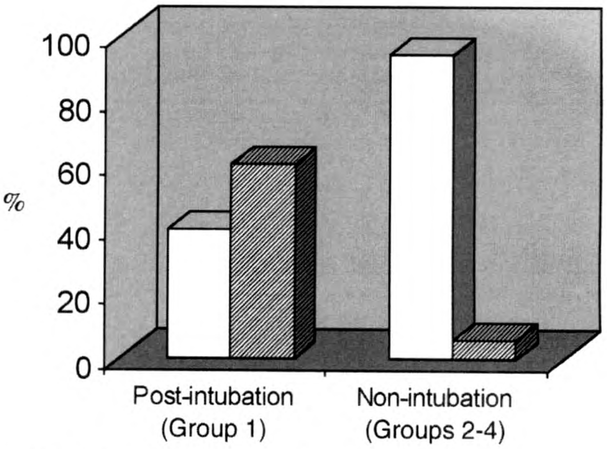

Fig 2. Distribution of patients according to sex and orotracheal intubation status. White bars — men; shaded bars — women.

da Laringe, São Paulo, over a 10-year period. The subjects were grouped according to the predominant cause: postintubation (group 1), vocal abuse (group 2), gastroesophageal reflux (group 3), and idiopathic causes (group 4). The sex of the patient was identified for each case in the 4 groups, and the ages ranged from 18 to 73 years. For the control group, 60 subjects (30 male and 30 female) without vocal complaints or lesions visible on telelaryngoscopy were randomly selected.

All subjects underwent a comprehensive otolaryngological assessment and videotelelaryngoscopy with a 70° rigid telescope (Machida, LY-C30) and a Toshiba charge-coupled device 1K-M 30 AK camera. Throughout the examination, the patients remained seated and received topical anesthesia with 10% lidocaine hydrochloride. The larynx was evaluated during inhalation for glottic proportion (GP) measurements[12]: the mathematical ratio between the midsagittal dimensions of the intermembranous and intercartilaginous portions of the glottis (Hirano[13]; Fig 1). In 9 cases GP was not measured because of technical difficulties, including an anterior commissure covered by the epiglottis,[1] the absence of identification of the vocal process tip, and incomplete glottic opening due to the reflex mechanism and muscular tension triggered by the examination. Among the 9 nonmeasured cases, 1 was a postintubation case, 5 were caused by vocal abuse, 2 were caused by GERD, and 1 had an idiopathic cause.

TABLE 1. DISTRIBUTION OF PATIENTS ACCORDING TO SEX AND PREDOMINANT CAUSE OF GRANULOMA

Group	*Men*	*Women*	*Total*	*%*
1 (postintubation)	6	9	15	22.7
2 (vocal abuse)	19	3	22	33.3
3 (gastroesophageal reflux disease)	20	0	20	30.3
4 (idiopathic)	9	0	9	13.7
Total	54	12	66	

The GP measurements were obtained by precise imaging of the glottic region in its widest opening during inhalation. Images of each examination were digitized with the video card model Spigot II Tape

TABLE 2. GLOTTIC PROPORTIONS FOR EACH GROUP

	Group 1	*Group 2*	*Group 3*	*Group 4*
	1.14*	1.17*	1.42	1.10
	1.00*	1.30*	1.21	1.19
	1.15*	1.28*	1.40	1.15
	0.90*	0.80	1.23	1.20
	1.11*	1.11	1.00	1.16
	1.00*	1.32	1.31	1.30
	1.20*	1.41	1.38	1.31
	1.14*	1.19	1.13	1.26
	1.10*	1.20	1.33	
	1.00	1.12	1.24	
	1.00	1.23	1.24	
	0.84	1.47	1.41	
	1.03	1.22	1.07	
	1.10	1.00	1.25	
		1.28	1.31	
		1.00	1.17	
		1.12	1.00	
			1.38	
Average	1.05	1.19	1.25	1.21

Glottic proportion is ratio between midsagittal dimension of intermembranous region and that of intercartilaginous region during inhalation.

*Female patients.

TABLE 3. AVERAGE GLOTTIC PROPORTIONS FOR POSTINTUBATION GROUP (GROUP 1) AND NONINTUBATION GROUPS (GROUPS 2-4)

	Group 1	*Groups 2-4*
Women	1.09	1.25
Men	1.10	1.28
Average	1.09	1.27

and the software Screen Play II connected to a personal computer. The pictures that displayed the maximum opening of the larynx during breathing were recorded on the computer's hard disk and were retrieved on the software Adobe Photoshop 3.0.4, with which GP measurements were made according to Fig 1.

The results obtained were used to compare the GP in the 4 groups and in the 2 control groups by statistical analysis. In order to check the differences between the groups, we conducted an analysis of variance. Then we carried out multiple Bonferroni tests to analyze the differences we had detected,[14] taking into account the significance level of .05.

RESULTS

The distribution of patients according to the predominant cause of granuloma (Table 1) was as follows: 15 postintubation cases (60% in women), 22 cases caused by vocal abuse (13.6% in women), 20 cases caused by GERD (0% in women), and 9 idiopathic granulomas (0% in women).

Figure 2 shows the distribution of patients according to sex and orotracheal intubation status; 60% of the postintubation group was female, compared to only 5.9% of the nonintubation group.

The GP averages were 1.05 for group 1, 1.19 for group 2, 1.25 for group 3, and 1.21 for group 4 (Table 2). The average for groups 2 through 4 (nonintubation) was 1.27 (Table 3). The averages for the control groups were 1.04 for female and 1.21 for male subjects (Table 4).

In the statistical analysis of GP carried out by multiple Bonferroni tests,[14] group 1 (postintubation) was similar to the control group of female subjects, and the values of both groups were statistically lower than those found for the control group of male subjects.

DISCUSSION

The distribution of patients according to cause of granuloma and sex agreed with the data reported in the literature,[2,4-7,9] in which female subjects are predominant in postintubation cases, and male subjects in all other causes. In our study, 94.1% of the nonintubation cases were in men (Fig 2).

TABLE 4. GLOTTIC PROPORTIONS FOR TWO CONTROL GROUPS

	Women	*Men*
	0.91	1.07
	0.91	1.08
	0.92	1.09
	0.95	1.09
	0.97	1.09
	0.99	1.09
	0.99	1.10
	0.99	1.10
	1.00	1.16
	1.00	1.16
	1.00	1.17
	1.00	1.18
	1.02	1.20
	1.03	1.21
	1.04	1.21
	1.04	1.21
	1.04	1.22
	1.04	1.22
	1.04	1.23
	1.05	1.23
	1.06	1.25
	1.12	1.26
	1.12	1.28
	1.14	1.29
	1.15	1.32
	1.17	1.35
	1.18	1.35
	1.18	1.41
	1.19	1.41
	1.20	1.44
Average	1.04	1.21

With regard to GP, statistical analysis showed that the groups were not similar (Tables 2-4). In fact, group 1 (postintubation) had a statistical performance similar to that of the control group of women, even though 40% of group 1 consisted of male subjects, and both groups were statistically lower in GP than the others. The midsagittal dimension of the intercartilaginous portion of the glottis had a value similar to that of the intermembranous portion (Fig 3A), contributing to an increased laryngeal susceptibility to trauma during intubation and the development of local granuloma at points of contact with the tube. Therefore, low values of GP, found mainly in women, were associated with postintubation granulomas, showing more susceptibility in women or in men with similar GP characteristics.

Groups 2 through 4, ie, groups without orotracheal intubation, had GP values statistically similar to each other and to that of the control group of men, with significantly higher values than group 1 or the female control group (Tables 3 and 4). These values

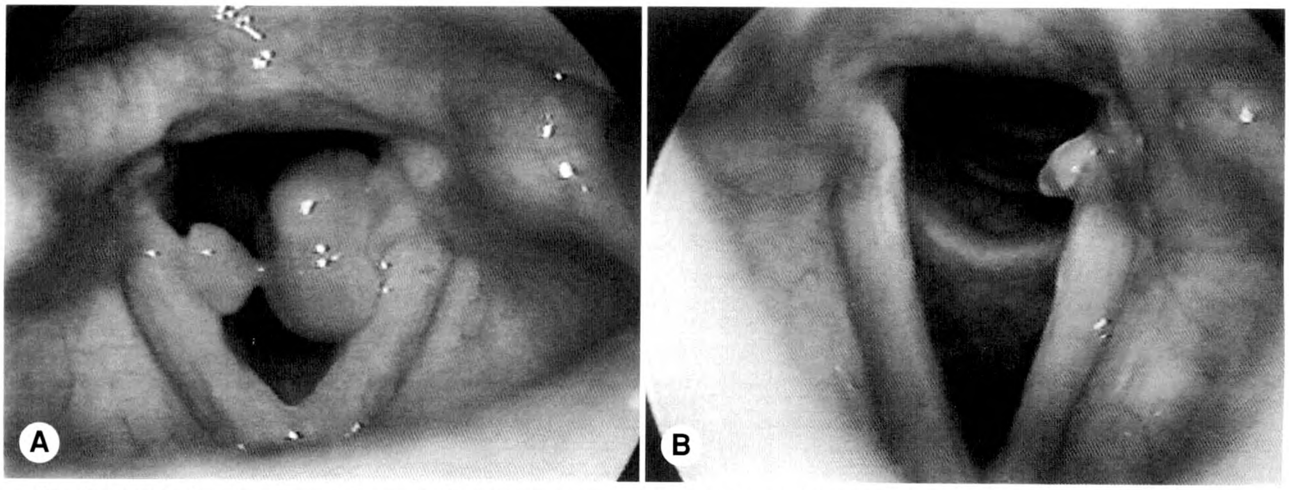

Fig 3. Posterior laryngeal granulomas. **A)** Related to orotracheal intubation. Midsagittal dimension of intercartilaginous portions had value similar to that of intermembranous portion. **B)** Not related to orotracheal intubation. Midsagittal dimension of intercartilaginous portion had lower value than that of intermembranous portion.

demonstrate that the dimension of the intermembranous region is proportionally larger in men (Fig 3B). Patients with granuloma are generally described as tense male business executives who frequently use hard glottal attacks.[9,15] Morrison and Rammage[16] described these as "muscle misuse voice disorders," in which there is a dysfunctional pattern in which the larynx is hyperadducted in a side-to-side manner. This misuse may account for an intense concentration of forces on the arytenoid region (Fig 4), where gastroesophageal reflux plays a role as an irritant and an aggravating factor in tensional trauma.

McFerran et al[3] reported that the predominance of granuloma in men was related to tension on the laryngeal region and the presence of hard voice onsets; it is known that these behaviors, as well as GERD, are not limited to men and may be found in women as well. The same authors agreed with the hypothesis of Snow et al[5] that the thinner female mucosa would support the formation of granuloma in postintubation cases; however, this hypothesis does not explain the male predominance in non–orotracheal intubation cases. Thinner mucosa, smaller size, and lower GP make the female larynx more susceptible to postintubation granulomas, despite the fact that granulomas are considered a rare complication of orotracheal intubation.[5] Nevertheless, regardless of these factors, the difference reflected by the GP protects the female larynx from the causes that predispose the male larynx to granulomas. In women, the occurrence of glottic coaptation with a triangular posterior chink is very high; this glottic configuration, reflected in GP values close to 1, protects the arytenoid region even when there is effort during vocal production.[13]

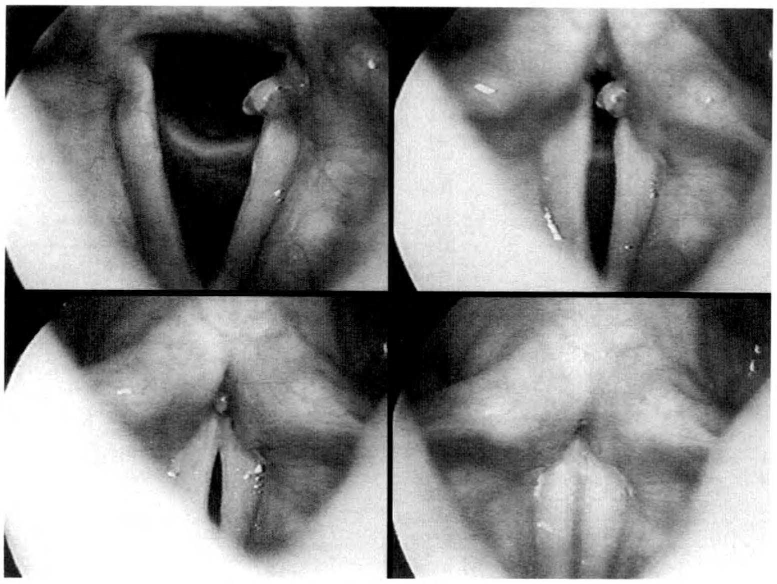

Fig 4. Vocal fold adduction and point of initial contact between vocal folds, highlighting impact on arytenoid region during phonation in male laryngeal pattern.

Consequently, we believe that the morphological configuration of the male larynx predisposes men to the formation of non–orotracheal intubation granulomas, because it turns the arytenoid region into the most impacted area during phonation. The female laryngeal morphology, with its lower GP, offers protection against the formation of granuloma during phonation, even in the event of laryngeal tension. Conversely, it makes female larynges more susceptible to post–orotracheal intubation granulomas.

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TRANSILLUMINATION LARYNGOSCOPY: A NEW WAY OF EVALUATING VOCAL FOLD AND HYPOPHARYNGEAL LESIONS

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Conventional methods of observing the larynx and the hypopharynx use reflected light to illuminate the larynx. The aim of this study was to see whether transilluminating the larynx was possible in subjects with and without disease. The larynx and the hypopharynx were observed by means of a rigid scope with a low-light charge-coupled device camera without the light guide inserted. Illumination was provided by a second rigid scope attached to a light source that was held at the neck by an assistant. The larynx and hypopharynx were observed by transillumination using both constant lighting and stroboscopy in 3 subjects with pharyngeal or laryngeal lesions and in 4 normal controls. The tumors were translucent or nontranslucent in appearance. A translucent polyp became nontranslucent when overlapping the vocal fold, thus indicating that a lesion the size of a polyp could be evaluated with this method. Mucosal waves could be observed during stroboscopy with transillumination, thus allowing observation of waves and lesions that cannot be observed with conventional stroboscopy.

KEY WORDS — laryngoscopy, stroboscopy, transillumination.

INTRODUCTION

It has been more than 150 years since Garcia's[1] observation of the larynx by indirect laryngoscopy, and more than 100 years has passed since stroboscopy was invented.[2] Although new techniques such as ultrahigh-speed cinematography[3] and, recently, ultrahigh-speed video imaging[4] and videokymography[5] have been developed, all of these techniques use reflected light for illumination and thus show only surface detail. Although a lesion such as a hard mass under the mucosa could interfere with the mucosal wave or show a discolored lesion and thus indicate an abnormal state, there are very few noninvasive ways to confirm it. Also, although pharyngeal lesions can be confirmed, there are very few methods available at an outpatient clinic that can evaluate the extent and texture of the lesion.

In this article, we show a method that could augment conventional means of evaluating vocal fold lesions and hypopharyngeal lesions. The method is relatively easy to perform, is not invasive, uses instruments that are readily available, and provides results that can be seen instantaneously.

MATERIALS AND METHODS

For the laryngeal observations of subjects without lesions, the larynges of 3 male volunteers (ages 35 to 60 years) and 1 female volunteer (age 34 years) were observed with both conventional and transillumination laryngoscopy. All of the normal subjects were healthy nonsmokers who had no history of laryngeal disease. The subjects and patients were of average build. The subjects with laryngeal and pharyngeal lesions were clinically examined by means of laryngoscopy and stroboscopy. After the conventional examinations, transillumination laryngoscopy was performed.

A black-and-white charge-coupled device (CCD) camera with a minimum sensitivity of 0.1 lux (RF Systems type MD-5, Nigata, Japan) was attached to

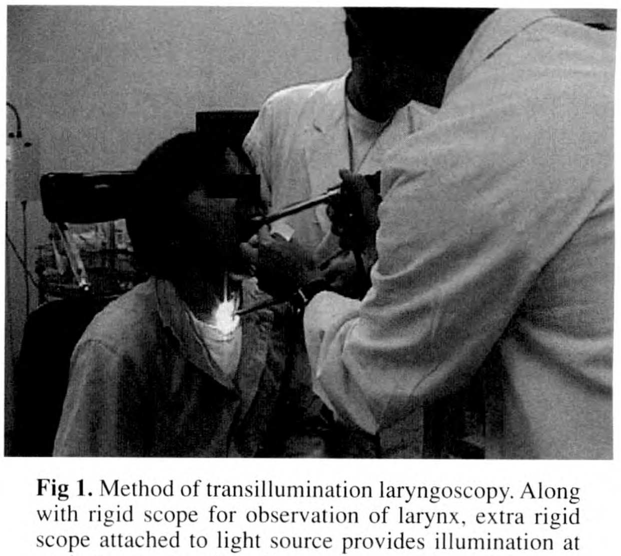

Fig 1. Method of transillumination laryngoscopy. Along with rigid scope for observation of larynx, extra rigid scope attached to light source provides illumination at neck above cricoid cartilage.

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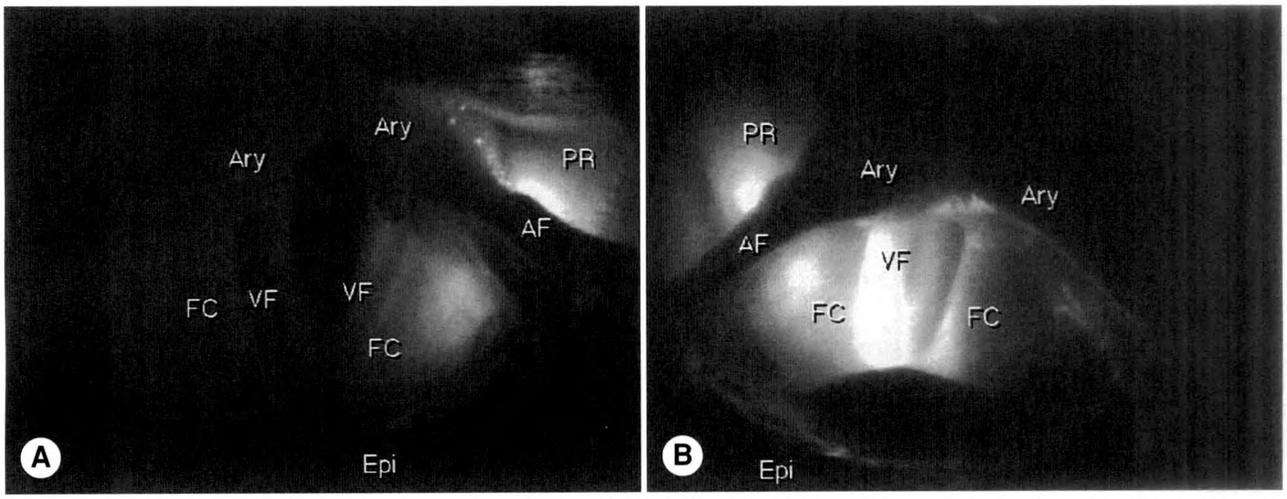

Fig 2. Views with transillumination laryngoscopy with constant light source. PR — pyriform recess, Ary — arytenoid, AF — aryepiglottic fold, VF — vocal fold, FC — false cords, Epi — epiglottis. **A**) Bubbles in pooling saliva can be seen in left pyriform recess. **B**) Illumination is slightly more medial than in A. Vocal fold can be seen more clearly.

a rigid endoscope (Nagashima Medical SFT-1, Tokyo, Japan). This endoscope was not connected to a light source. A second rigid endoscope, which provided the illumination, was connected to either an ordinary constant light source (Nagashima Medical NX150F) or a stroboscope (Nagashima Medical LS-3A). The endoscope with the camera was inserted into the oral cavity as for conventional laryngoscopy.

An assistant held the second rigid endoscope, which was attached to the light source, against the neck, above the cricoid cartilage (Fig 1). The tip of the endoscope was held perpendicular to the surface at the midline of the neck. When necessary, the endoscope was moved laterally or medially while the tip was held perpendicular against the surface of the skin. Because the light source generates heat from the tip of the endoscope, it is not advisable to keep the illuminating endoscope in the same position on the skin for prolonged periods. Our method is to wait until the observing endoscope is in the correct position in the oral cavity before applying the second endoscope to the neck for illumination. Heat generation was a problem with the constant light source; in contrast, the stroboscopic light source presented only minimal problems with heat.

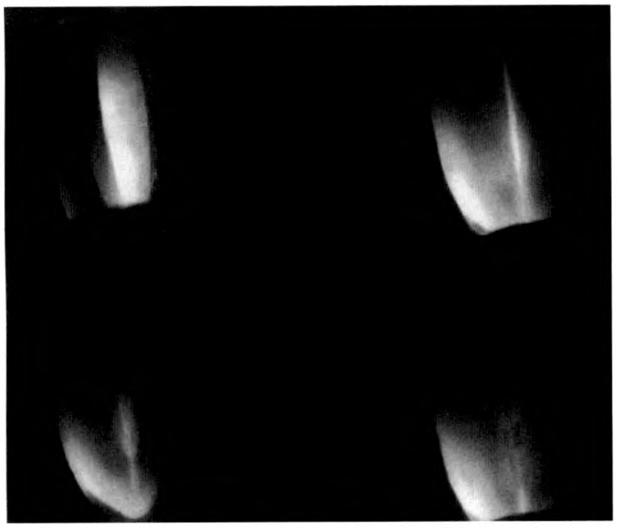

Fig 3. Transillumination laryngoscopy with stroboscopy. Vibrating mucosa can be seen. Dark spots at middle of vocal folds in lower right photo are sputum.

The images were recorded with an s-VHS tape recorder (Panasonic AG7355, Matsushita Electric Industrial Co, Tokyo) and printed on a video printer (Sony UP-2900MD, Tokyo) or captured on a personal computer via a still capture device. Other than the CCD camera, the instruments were ordinary ones used in routine outpatient clinical setups.

RESULTS

It was possible to view the larynges of all 4 normal control subjects with transillumination laryngoscopy. Figures 2 and 3 show the normal larynx of a 35-year-old man by means of stroboscopy and a constant light source. With the constant light source, not only could the vocal fold be observed, but even bubbles in a small amount of pooling saliva within the left pyriform recess could be seen (Fig 2A). The angle of the illuminating rigid scope determines the area that can be transilluminated. Figure 2B shows a slightly more medial area being transilluminated in the same subject. The vocal fold is more visible in this figure, but the pyriform recess is not.

By stroboscopy with transillumination, the mucosal wave could be seen. Figure 3 shows a series of vibrating vocal folds illuminated by this method.

Case 1. Patient 1 was a 28-year-old woman with a hypopharyngeal mass. On conventional laryngos-

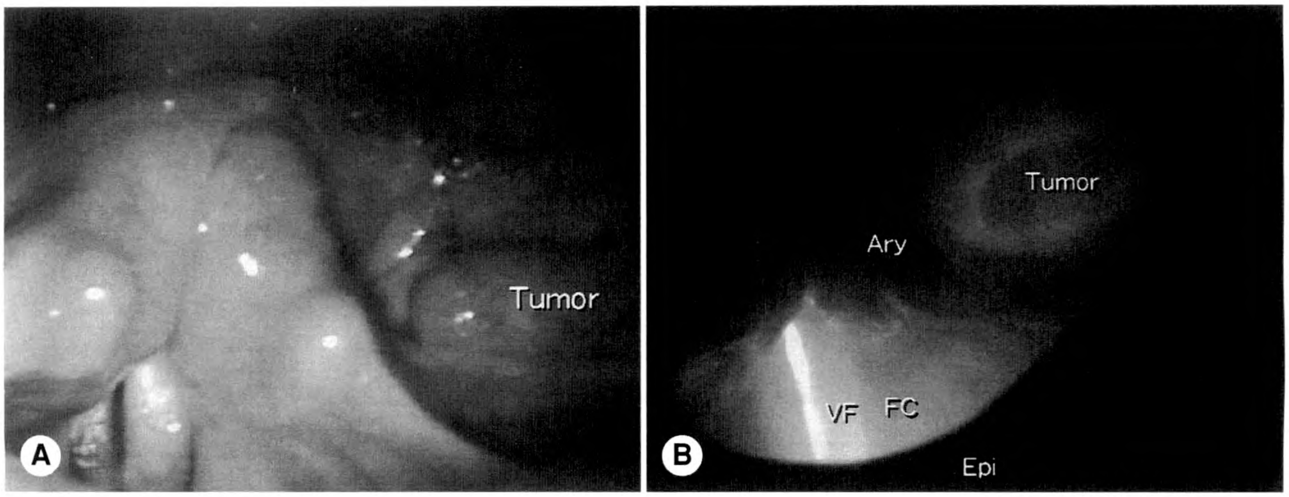

Fig 4. (Case 1) **A)** Hypopharyngeal mass. Oval-shaped mass can be seen in left pyriform recess. **B)** Hypopharyngeal mass viewed with transillumination laryngoscopy. Extent of lesion can be clearly seen in left pyriform recess. See Fig 2 for abbreviations.

copy, a tumor with a smooth surface was seen in the left pyriform recess (Fig 4A). With transillumination laryngoscopy, the tumor was seen to be nontranslucent and to have a well-defined edge (Fig 4B). Because the lesion was thought to be benign and the patient refused a biopsy, the patient is being followed up in the outpatient clinic.

Case 2. Patient 2 was a 38-year-old woman with a polyp and a cyst. On conventional laryngoscopy, the polyp was seen at the free edge of the right vocal fold. Conventional stroboscopy revealed that the mucosal wave was absent in the middle of the left vocal fold. On transillumination laryngoscopy, the polyp seemed translucent (Fig 5A), but when it overlapped the right vocal fold, it appeared nontranslucent (Figs 5B and 6). There was also a nontranslucent area in the middle of the left vocal fold that seemed to correspond to a cyst. Transillumination stroboscopy confirmed the lesion by showing that the mucosal wave was disrupted at the middle of the vocal fold. The motion was similar to that of a wave at the beach with a rock in its path. The mucosal wave was broken into 2 sections at the site of the cyst, and there was no wave at the site of the cyst. Surgery was performed, the existence of the cyst was confirmed, and the cyst was removed along with the polyp.

Case 3. Patient 3 was a 49-year-old man with a huge mass under the mucosa from the left pyriform recess to the left vestibular fold (Fig 7A). Because transillumination laryngoscopy showed a translucent mass with a relatively well-defined edge, the most probable diagnosis was thought to be a cyst.

Figure 7B shows the translucent image. The blood vessels of the tumor could be seen by transillumination at the left vestibular fold area. Surgical biopsy showed a solid mass under the mucosa, and the pathological diagnosis was a neurinoma.

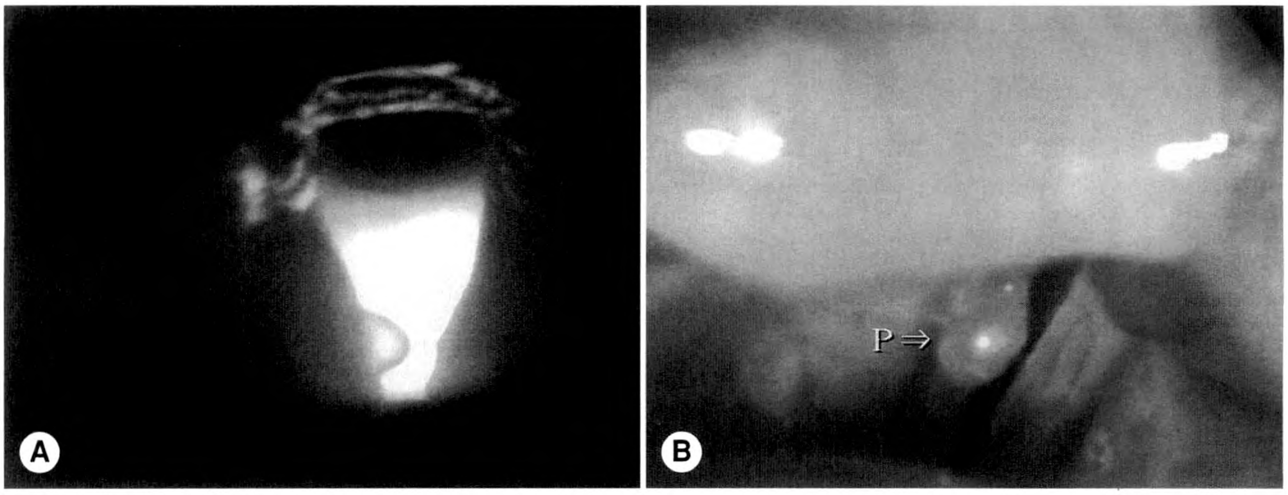

Fig 5. (Case 2) **A)** Polyp is not overlapping vocal fold. Polyp appears translucent. **B)** Polyp (P) is overlapping right vocal fold. Observation by conventional laryngoscopy.

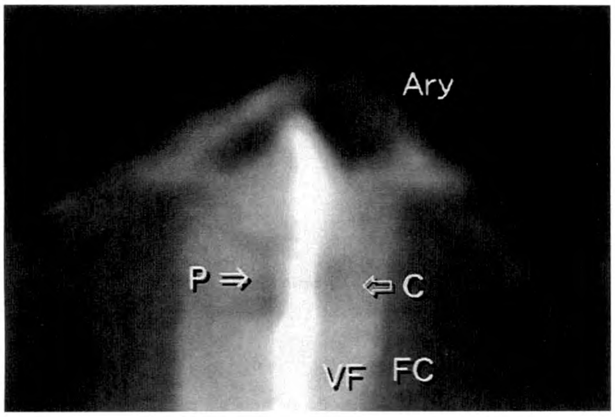

Fig 6. (Case 2) Polyp is overlapping right vocal fold. Observation by transillumination laryngoscopy. Polyp appears nontranslucent. There is also nontranslucent area in left vocal fold that seems to correspond to cyst (C).

DISCUSSION

In normal patients, the larynx and hypopharynx could be seen with relative ease with transillumination laryngoscopy, and it was thought that it would not be a difficult task to apply this method to patients with lesions. Although observation with transillumination laryngoscopy was not difficult, interpretation was difficult, as seen in case 3. Although more data are needed to correlate the type of lesion with the data obtained by transillumination, this method seems to hold some promise. The fact that in case 2, the polyp was not translucent when it overlapped the vocal fold means transillumination laryngoscopy could reveal a lesion as small as this 1- to 2-mm polyp. Also, although it is not possible with normal stroboscopy to see a mucosal wave that is hidden by overhanging vocal fold mucosa, with transillumination stroboscopy it was possible to see the mucosal wave distortion created by an embedded vocal fold lesion.

Stroboscopic views with transillumination laryngoscopy could reveal lesions that have a translucency different from that of the surrounding tissue. It is difficult to observe a lesion embedded under the mucosa with a conventional stroboscopic view; the only evidence of such a lesion is a missing mucosal wave. With transillumination laryngoscopy, both the missing wave and the actual extent of the lesion could be observed.

The type of light source and type of CCD camera used affect the images seen during transillumination laryngoscopy. In general, because light waves of longer length go through thicker tissue, a CCD camera with more infrared sensitivity would have brighter images, and conversely, a light source without infrared filters, or with more red or infrared light spectrum output, would have more translucent light. For this reason, it is important to include a notation of the type of camera and type of light source with any picture obtained by transillumination laryngoscopy. Preliminary studies with a color CCD camera confirm this conclusion. Only light of longer wavelengths is seen, so that the image is almost totally red. The effect that the type of light source and type of CCD camera have on the image has not yet been quantitatively measured, although it is currently being evaluated. The reason for this delay is that acquisition of multiple light sources and standardization of procedures have been difficult.

This method could be difficult in heavy subjects with thick necks. Although we had success in observing mildly obese patients, severely obese patients could present a challenge. A thick neck would block light, and the translucent light would not be observable. We had 1 or 2 cases out of more than 40 patients in which the neck thickness was a problem, and changes in positioning seemed to help.

We are currently using a rigid endoscope to pro-

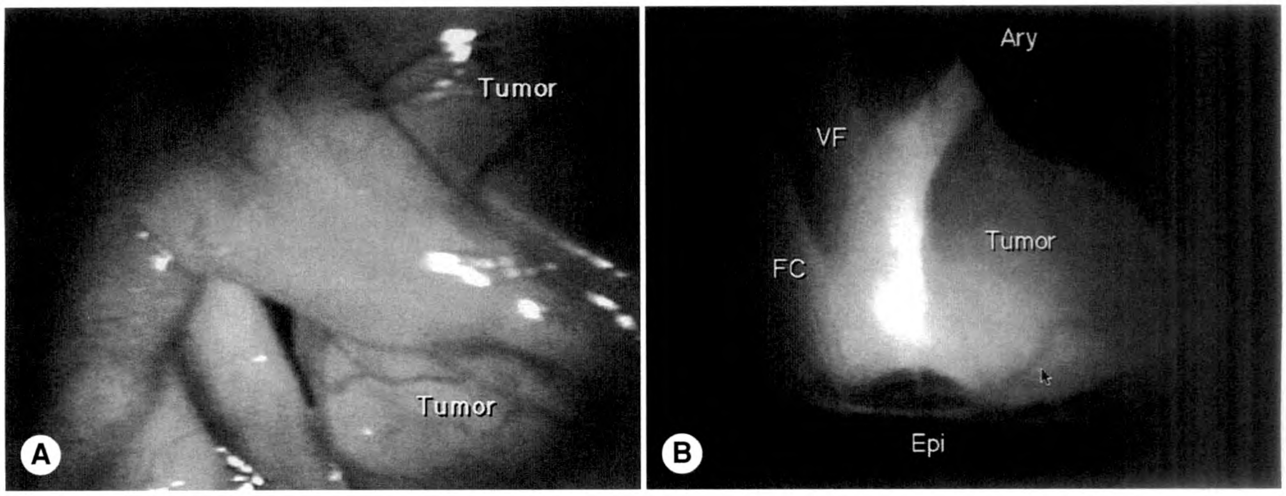

Fig 7. (Case 3) **A**) Laryngeal tumor extending from left pyriform recess to left vestibular fold area. **B**) By transillumination laryngoscopy, mass appears translucent at left vestibular fold area. Blood vessels (arrow) can be seen by transillumination.

vide illumination, but other instruments could be equal or superior to our method. We have used fiber-optically guided headlights to transilluminate the larynx, and although the amount of light was sufficient, the rigid scope was easier to use, because the angle and length of the rigid scope allow the assistant to stand at the side and not in the way of the examiner. Of course, a custom-made transillumination device could have more light fibers than a rigid scope, and could be brighter and easier to set up, but it would be more expensive and difficult to acquire. If this method gains popularity, such a device might be marketed in the future. However, one of the best points of this method is that the only device required, besides the routine otolaryngological instruments, is the inexpensive CCD camera.

CONCLUSION

Although more work is needed to make this method more useful, we believe that transillumination laryngoscopy adds information that is not readily available by conventional methods, and that it could be useful for the diagnosis of laryngeal and hypopharyngeal lesions.

ACKNOWLEDGMENTS — The authors thank Dr Suguru Fukaya for providing the time to work on this paper, and Dr Dorothy E. Ross for assistance in the preparation of the manuscript.

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COMPARISON OF CYFRA 21-1 AND SQUAMOUS CELL CARCINOMA ANTIGEN IN DETECTING NASOPHARYNGEAL CARCINOMA

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CYFRA 21-1 is a newly developed tumor marker that is especially useful for detecting squamous cell carcinoma (SCC) of the lung. Squamous cell carcinoma antigen is a proven tumor marker that is especially useful for detecting SCC of the cervix. Our aim in this study was to compare the clinical value of CYFRA 21-1 and SCC antigen in the detection of nasopharyngeal carcinoma (NPC). Serum levels of CYFRA 21-1 and SCC antigen were measured in 80 untreated NPC patients and 77 healthy controls. The cutoff values of CYFRA 21-1 and SCC antigen, determined at the 95th percentile of the 77 healthy controls, were 2.48 ng/mL and 1.49 ng/mL, respectively. The results revealed that the mean serum value of only CYFRA 21-1 was significantly higher in the 80 NPC patients than in the 77 healthy controls, and the detection sensitivity of CYFRA 21-1 for NPC was significantly higher than that of SCC antigen. In conclusion, our results suggest that CYFRA 21-1 is a better tumor marker than SCC antigen for detection of NPC.

KEY WORDS — CYFRA 21-1, nasopharyngeal carcinoma, squamous cell carcinoma antigen.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a very common cancer in both men and women in southern China and Taiwan.[1,2] There are no early symptoms of NPC, because it grows in a hidden anatomic region, and hence, the disease is difficult to diagnose. At present, the value of serum tumor markers for detecting NPC is still questionable, and the search for a useful marker is worthwhile. Squamous cell carcinoma (SCC) antigen was first introduced by Kato and Torigoe[3] in 1977 and was found useful for detecting SCC in the uterine cervix. Histologically, SCC antigen is logically supposed to be sensitive in detecting SCC.[4,5] Also, a fragment of cytokeratin subunit 19 (CYFRA 21-1) can be measured in serum by a new immunoradiometric assay. Previous studies have proven that serum CYFRA 21-1 is a more useful tumor marker than SCC antigen for detecting SCC of the lung.[6,7] CYFRA 21-1 also has good sensitivity for detecting other SCCs.[5-7] The aim of our study was to compare the detection sensitivities of CYFRA 21-1 and SCC antigen in NPC patients.

MATERIALS AND METHODS

Serum levels of CYFRA 21-1 and SCC antigen were measured in 80 patients (23 women, 58 men; age range, 27 to 80 years) with untreated NPC confirmed by pathological biopsy findings. Fifty-eight cases (72.5%) were SCC, and 22 cases (27.5%) were undifferentiated carcinoma. Tumor extent and nodal involvement were assessed by a complete physical examination, a detailed head and neck evaluation, computed tomography or magnetic resonance imaging of the head and neck, technetium 99m methylene diphosphonate whole-body bone scanning, and chest X-ray. All 80 NPC patients were classified according to the International Union Against Cancer (UICC) TNM classification system.[8] One case was classified as T1, 46 cases as T2, 8 cases as T3, and 25 cases as T4. Seventeen cases were classified as N0, 5 cases as N1, 48 cases as N2, and 10 cases as N3. One case was stage I in the TNM classification system, 10 cases were stage II, 7 cases were stage III, and 62 cases were stage IV. In addition, 77 healthy subjects (22 women, 55 men; age range, 25 to 75 years) without evidence of neoplasm were included as controls.

A blood sample was collected from each NPC patient before any treatment and from each healthy control subject. All samples were separated, and the sera were frozen and stored at –20°C until testing. According to the manufacturer's instructions, CYFRA 21-1 and SCC antigen serum levels were measured with a solid-phase immunoradiometric assay using monoclonal antibodies (CIS bio-International, Paris, France, and Abbott Laboratories, Abbott Park, Illinois).

Independent Student's *t*-tests were used to evaluate the statistical differences in the means of CYFRA

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TABLE 1. LEVELS OF CYFRA 21-1 AND SCC ANTIGEN IN NORMAL CONTROLS AND NPC PATIENTS

						Median		Range		95th Percentile	
	No. of Cases	CYFRA 21-1 (mean ± SD)	p	SCC Antigen (mean ± SD)	p	CYFRA 21-1	SCC Antigen	CYFRA 21-1	SCC Antigen	CYFRA 21-1	SCC Antigen
Healthy controls	77	1.31 ± 0.56		0.71 ± 0.44		1.11	0.66	0.78-3.35	0.06-2.21	2.48	1.49
NPC patients	80	6.21 ± 10.32	<.05*	0.81 ± 0.76	>.05	2.99	0.58	0.93-74.11	0.02-3.34	18.04	2.11
Pathological findings of NPC											
SCC	58	6.63 ± 11.81		0.86 ± 0.83		3.10	0.63	0.93-74.11	0.02-3.34	30.99	2.89
Undifferentiated carcinoma	22	5.10 ± 4.44	>.05	0.66 ± 0.50	>.05	2.79	0.48	1.09-14.80	0.11-1.84	13.20	1.62
T stage of NPC											
T1-2	47	4.07 ± 5.08		0.76 ± 0.64		2.52	0.60	0.93-31.94	0.04-3.31	12.39	1.93
T3-4	33	9.26 ± 14.47	<.05*	0.87 ± 0.90	>.05	4.39	0.57	0.99-74.11	0.02-3.34	30.99	2.89
N stage of NPC											
N0-1	22	2.48 ± 1.85		0.58 ± 0.57		1.90	0.33	0.99-9.27	0.02-2.06	4.68	1.93
N2-3	58	7.63 ± 11.78	<.05*	0.89 ± 0.80	>.05	3.73	0.65	0.93-74.11	0.03-3.34	30.99	2.89
TNM stage of NPC											
I-III	18	2.64 ± 1.96		0.67 ± 0.59		1.98	0.48	1.09-9.27	0.04-2.06	4.68	1.93
IV	62	7.25 ± 11.48	>.05	0.85 ± 0.80	>.05	3.44	0.61	0.93-74.11	0.02-3.34	30.99	2.89

Data are in nanograms per milliliter.

SCC — squamous cell carcinoma, NPC — nasopharyngeal carcinoma.

*Statistically significant.

21-1 and SCC antigen serum levels between the healthy controls and the NPC patients, as well as between the subgroups of NPC patients with different pathological findings and stages. If the p value was less than .05, the differences were considered significant. The cutoff values for CYFRA 21-1 and SCC antigen, determined at the 95th percentile of the standard gaussian variate of the 77 healthy controls, were 2.48 ng/mL and 1.49 ng/mL, respectively. If the serum levels of CYFRA 21-1 or SCC antigen in NPC patients were less than 2.48 ng/mL or 1.49 ng/mL, respectively, the results were considered positive. Either χ^2 or Fisher's exact tests were used to evaluate the statistical differences in the detection sensitivities between CYFRA 21-1 and SCC antigen for all NPC patients and their subgroups. If the p value was less than .05, the differences were considered significant.

RESULTS

The detailed data are listed in Tables 1 and 2. The mean serum value of CYFRA 21-1 in all 80 NPC patients was significantly higher than that in the 77 healthy controls ($p < .05$), but no significant difference for SCC antigen was found. There was no significant difference in CYFRA 21-1 and SCC antigen serum values between the SCC patients and patients with undifferentiated carcinoma ($p > .05$). There were significant differences in CYFRA 21-1 serum levels and detection sensitivities between T1-2 and T3-4 patients, and between N0-1 and N2-3 patients. There

TABLE 2. DETECTION SENSITIVITIES OF CYFRA 21-1 AND SCC ANTIGEN IN NPC PATIENTS

	No. of Cases	No. of Cases With Positive Results		Detection Sensitivity (%)		p
		CYFRA 21-1	SCC Antigen	CYFRA 21-1	SCC Antigen	
NPC patients	80	47	12	58.8	25.5	<.05*
Pathological findings of NPC						
Moderately or poorly differentiated SCC	58	35	10	60.3	28.6	<.05*
Undifferentiated carcinoma	22	12	2	54.5	16.7	<.05*
T stage of NPC						
T1-2	47	25	6	53.2	24.0	<.05*
T3-4	23	22	6	95.7	27.3	<.05*
N stage of NPC						
N0-1	22	7	2	31.8	28.6	>.05
N2-3	58	40	10	69.0	25.0	<.05*
TNM stage of NPC						
I-III	18	6	2	33.3	33.3	>.05
IV	62	41	10	66.1	24.4	<.05*

*Statistically significant.

were no significant differences in SCC antigen serum levels and detection sensitivities between T1-2 and T3-4 patients, between N0-1 and N2-N3 patients, or between TNM I-III and TNM IV patients ($p > .05$). The detection sensitivity of CYFRA 21-1 for all 80 NPCs was significantly higher than that of SCC antigen ($p < .05$). The detection sensitivities of CYFRA 21-1 for SCC, undifferentiated carcinoma, and T1-2, T3-4, N2-3, and TNM IV lesions were significantly higher than those of SCC antigen ($p < .05$).

DISCUSSION

Tumor markers are useful to clinicians for screening asymptomatic patients for the presence of cancer, for establishing a new cancer diagnosis, for predicting patients' prognoses, for monitoring the response to therapy, and for detecting cancer recurrence early. Many tumor markers have proven to be clinically helpful in various human cancers. However, a reliable serum tumor marker for NPC remains elusive. In 1977, Kato and Torigoe[3] first reported detecting a tumor-associated antigen in patients with SCC of the uterine cervix. Using a radioimmunoassay, they detected the antigen in 77% of 35 patients with cervical SCC. Since then, SCC antigen has become a useful tumor marker for detecting SCC in different cancers. However, the use of SCC antigen in patients with head and neck SCC has been unsatisfactory. In previous studies, the diagnostic sensitivity of SCC antigen for head and neck SCC ranged from 19% to 47.5%.[4,5,9,10] In accordance with previous study results and with our own findings (Table 2), therefore, we consider SCC antigen to be of limited value in detecting NPC.

As main components of the cell cytoskeleton, cytokeratins are proteins of the intermediate filament family. They are expressed by all epithelial cells and appear to be useful markers of epithelial differentiation.[11-13] Cytokeratin 19 (CYFRA 21-1) has a low molecular weight of 40 kd and is expressed particularly in epithelial tumor cells.[12,14] For these reasons, the cytokeratin subunit 19 fragment can be considered a tumor marker for carcinoma. In our study, the CYFRA 21-1 serum levels in NPC patients were significantly higher than those in controls. In addition, the detection sensitivity of CYFRA 21-1 for NPC was higher than that of SCC antigen. Although the sensitivity of CYFRA 21-1 for SCC was slightly higher than that for undifferentiated carcinoma, there was no significant difference between these two different pathological types of NPC. This finding differs from the results of CYFRA 21-1 in detection of lung cancer, which showed significantly higher detection sensitivities for SCC than for other pathological types of lung cancer.[15,16] In addition, we found a significantly higher CYFRA 21-1 serum level and a significantly higher detection sensitivity for more advanced NPC (T3-4, N2-3, and TNM IV; Tables 1 and 2).

We conclude that CYFRA 21-1 is a better tumor marker for detecting NPC than is SCC antigen. The serum CYFRA 21-1 level may have a potential clinical role as a helpful test in monitoring therapy response and detecting NPC recurrence. However, further investigation is recommended to verify this hypothesis.

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MANAGEMENT OF THE TINNITUS PATIENT

The Ninth Annual Conference on the Management of the Tinnitus Patient, for professionals and tinnitus patients, will be held in Iowa City, Iowa, October 4-6, 2001. For information, see web site www.medicine.uiowa.edu/otolaryngology/news/news or contact Rich Tyler by telephone (319-356-2471) or e-mail (rich-tyler@uiowa.edu).

Ann Otol Rhinol Laryngol 110:2001

MORPHOLOGICAL AND HISTOCHEMICAL STUDIES OF THE GENIOGLOSSUS MUSCLE

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The purpose of this preliminary study was to assess the histo-anatomic composition of the genioglossus muscle fibers. The genioglossus muscles were obtained from 4 cadavers and 1 autopsy specimen. On morphological study, the average diameters of the muscle fibers were seen to gradually increase, from the fibers that ran anteriorly to the dorsum of the tongue, to the fibers that ran posteriorly to the root of the tongue. Histochemical study revealed that type II fibers were significantly predominant in the anterior portion; there was no dominant fiber type in the posterior portion. Gradual changes in diameter were independent of fiber type. These findings may suggest that the fibers of the anterior portion are suitable for phasic action, and that the posterior is relatively tonic; and the posterior has larger absolute muscle strength than the anterior. It is thought that the fibers of the posterior portion might contribute to the maintenance of the mesopharyngeal airway and to vowel production, and that the anterior fibers might contribute to some fine movements and to consonant production.

KEY WORDS — genioglossus muscle, histochemistry, morphology, myosin adenosine triphosphatase.

INTRODUCTION

The tongue has been studied for years as a speech organ. The various methods — X-ray cineradiography,[1,2] the X-ray microbeam system,[3,4] ultrasound imaging,[5-7] tagging snapshot magnetic resonance imaging,[8,9] electromyography,[10-13] and so on — are still useful, but they indicate only surface or general morphological changes of the tongue. Among these methods, electromyography is the most useful for investigating the function of a target muscle. The structure of the tongue is complicated, however, because the intrinsic and extrinsic tongue muscles cross each other in the tongue.[14] Therefore, we cannot examine how each muscle of the tongue functions in detail by the electromyographic approach. In order to investigate the function of each muscle of the tongue, we selected an anatomic procedure, because it is thought that the anatomic findings of muscles in the head and neck, especially morphological and histochemical findings, are a close reflection of function.[15-19]

The genioglossus muscle is one of the extrinsic tongue muscles; it originates from the mental spine of the mandible and spreads out radially to the dorsum of the tongue. There have been many studies of the genioglossus muscle by electromyographic approaches in various fields. It has been reported that the genioglossus muscle plays a large role in shaping the vowels.[10-13] Smith[11] reported that the activities of different portions of the genioglossus muscle differed according to the vowel. Thus, some speech scientists have thought that the genioglossus muscle consisted of a number of components functionally, rather than acting as "one muscle."[12]

On the other hand, from studies of sleep apnea, the genioglossus muscle is known to be responsible for the maintenance of the upper airway in the supine position.[20,21] Therefore, the genioglossus muscle has been labeled a "safety muscle."

Thus, the genioglossus muscle has both a phasic function as a speech organ and a tonic one in the respiratory tract. Where do these functions of the genioglossus muscle originate? The purpose of this study was to investigate the genioglossus muscle with morphological and histochemical approaches.

MATERIALS AND METHODS

Four tongues were obtained from human cadavers that had been fixed in 4% formaldehyde solution for more than 6 months, and 1 was obtained from an autopsy of a 65-year-old man with no speech disorders who had been dead less than 4 hours. All tongue

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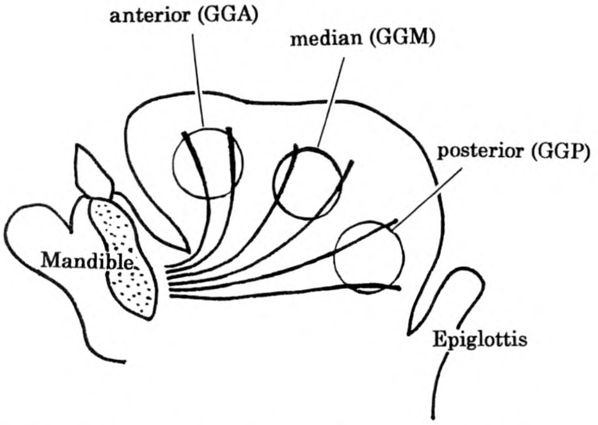

Fig 1. Three portions of genioglossus muscle. GGA — anterior portion, from which fibers run anteriorly to dorsum of tongue; GGP — posterior portion, from which fibers run posteriorly to root of tongue; GGM — median portion, in which fibers run between GGA and GGP.

samples were removed by total glossectomy by the subperiosteal pull-through technique.

The 4 cadaver samples were cut in half sagittally. We then selected and cut off 3 portions of the genioglossus muscle that were not crossed by any other tongue muscle, for example, the transverse intrinsic muscle. The 3 portions were the anterior portion (GGA), in which the fibers ran anteriorly to the dorsum of the tongue, the posterior portion (GGP), in which the fibers ran posteriorly to the root of the tongue, and the median portion (GGM), in which the fibers ran between the GGA and the GGP (Fig 1). After embedding of these specimens with paraffin, sections of the 3 portions were cut in series at 10 μm. For light microscopy, the sections were stained with hematoxylin and eosin. Then, the circumferential length was measured on 200 fibers from each sample by the NIH imaging system. The circumferential length was converted to a presumed round circumference, and diameters were obtained from the presumed round circumference.[18,22]

The autopsy sample was fixed immediately in 2% glutaraldehyde solution for less than 30 minutes. Two portions obtained from the anterior portion (GGA) and the posterior portion (GGP) of the genioglossus muscle were cut off. Two specimens were frozen in cooled acetone with dry ice. Sections were cut in series at 5 μm in a cryostat. For histochemical study, the sections were stained with a myosin adenosine triphosphatase (ATPase) reaction after both alkaline preincubation (pH 10.5) and acid preincubation (pH 4.2).[23,24] The samples to which the ATPase reaction was applied after acid preincubation presented type I fibers with heavy staining and type II fibers with weaker staining. The samples with alkaline preincubation presented the reverse. The numbers of each

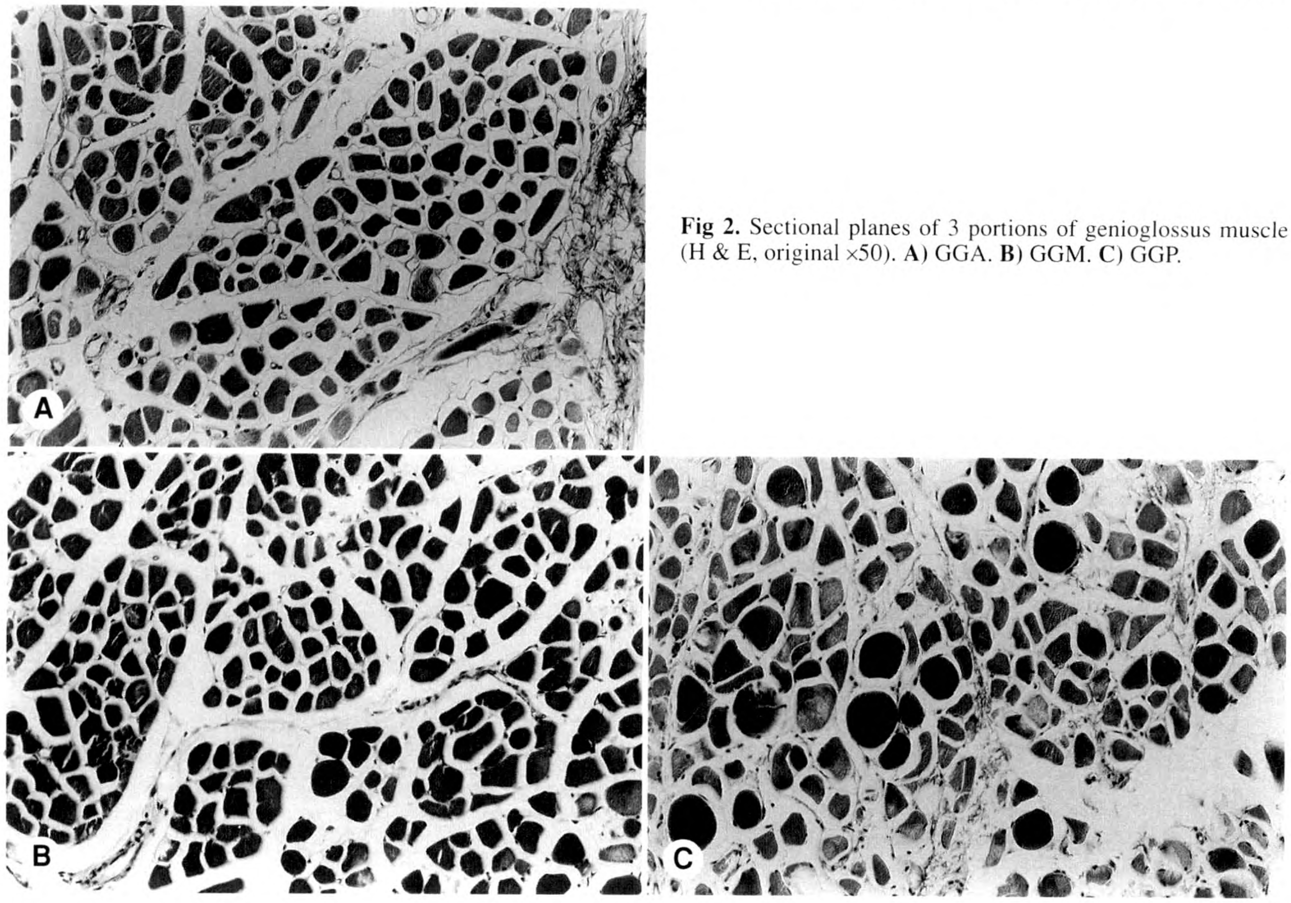

Fig 2. Sectional planes of 3 portions of genioglossus muscle (H & E, original ×50). **A**) GGA. **B**) GGM. **C**) GGP.

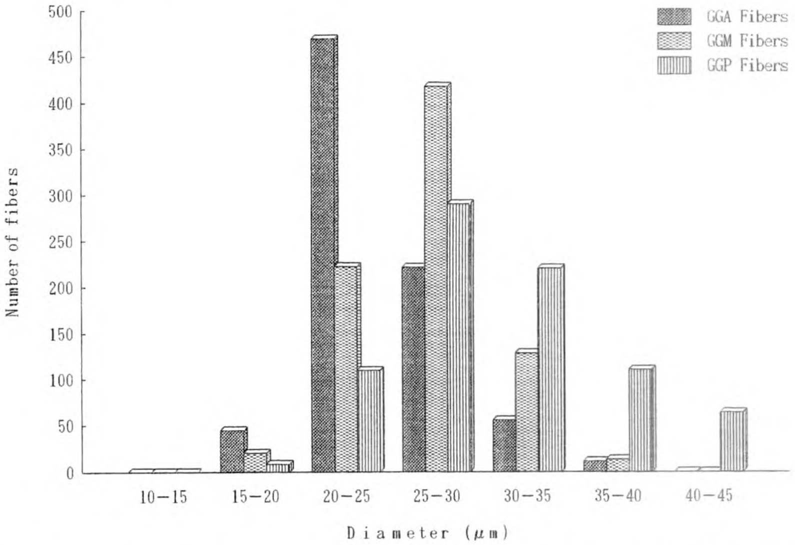

Fig 3. Distribution of fiber diameters according to 3 portions of genioglossus muscle. Fibers of GGA were smaller-diameter fibers in general. Fibers of GGP were larger, and GGM fibers were in middle, between GGA and GGP.

fiber type were counted on 200 fibers for each sample. The diameters of each type of fiber were also measured in the same way.

RESULTS

Distribution of Fiber Diameters. The 4 samples showed similar results. Light microscopy showed a clear difference in the diameters of the 3 portions (Fig 2). Figure 3 is a megahistogram of the distributions of fiber diameters for the 3 portions in the 4 samples. The total number of fibers for each portion was 800. The fibers of the GGA were generally small-diameter fibers, ie, 12.4 to 32.2 μm (average, 19.2 ± 3.5 μm). The GGM had small- and medium-diameter fibers, ie, 13.9 to 31.7 μm (average, 21.8 ± 3.7 μm). The GGP had small, medium, and large fibers, with a range of 16.7 to 38.9 μm (average, 25.7 ± 5.3 μm). The differences in diameter among the GGA, GGM, and GGP were significant, with the average diameter of the GGP being larger than that of the GGM, and that of the GGM being larger than that of the GGA ($p < .05$; Table 1).

Distribution of Fiber Types. For the GGA, in the ATPase reaction after alkaline preincubation at pH 10.5, type I fibers represented 31.3% of the population and type II fibers 68.7%, and after acidity preincubation at pH 4.2, type I fibers represented 32.5% and type II fibers 67.5% (Fig 4). For the GGP, in the ATPase reaction after alkaline preincubation at pH 10.5, type I fibers represented 51% and type II fibers 49%, and after acidity preincubation at pH 4.2, type I fibers represented 48.9% and type II fibers 51.1% (Fig 5). Because the ATPase reactions after both alkaline and acid preincubations gave almost similar distributions for muscle fiber types of the autopsy samples, we were confident of the results. In the ATPase reaction after alkaline preincubation, the type I fibers of the GGA presented an average diameter of 24.5 ± 5.2 μm, and type II fibers were 21.0 ± 6.5 μm. In contrast, the type I fibers of the GGP presented an average diameter of 29.6 ± 6.5 μm, and the type II fibers were 34.6 ± 9.0 μm (Table 2). The differences in diameter of both muscle types between the GGA and the GGP were significant, with the average diameter of the GGP being larger ($p < .05$). The diameters of the types that were obtained from the GGA had no significant difference. The difference in diameter between type I and type II fibers in the GGP was significant, with type I being smaller than type II ($p < .05$).

TABLE 1. SIZE OF FIBERS OF THREE PORTIONS OF GENIOGLOSSUS MUSCLE

Portion	*Fiber Diameter (μm)*	
Anterior	19.2 ± 3.5	
		*
Median	21.8 ± 3.7	
		*
Posterior	25.7 ± 5.3	

Data are mean ± SD.
*$p < .05$.

DISCUSSION

The genioglossus muscle is the largest muscle of the tongue. It originates from the genial tubercle on the lingual surface of the mandible. Its fibers run posteriorly in a radial fashion toward the dorsum and the root of the tongue. The genioglossus muscle greatly contributes to the morphological changes of the tongue during the production of vowels; in particular, it appears to "bunch" the tongue into a position suitable for high front vowels.[10-13] Functionally distinct components of the muscle have been postulated by Heffner,[10] ie, anterior and posterior components. A similar pattern was noted on electromyographic studies by Miyawaki et al[12] for Japanese vowels and

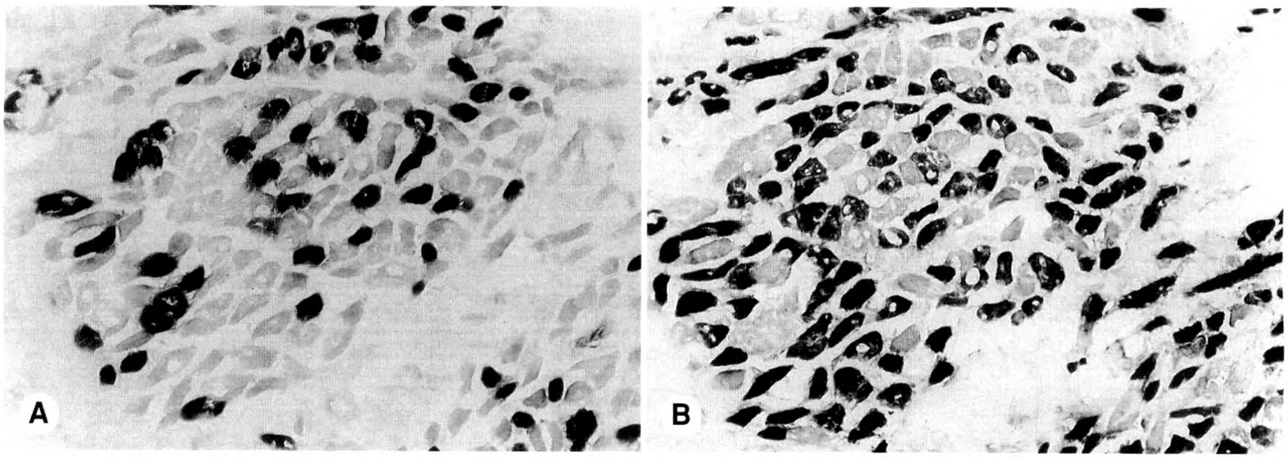

Fig 4. GGA stained by myosin adenosine triphosphatase reaction (original ×50). **A)** At pH 4.2. **B)** At pH 10.5.

by Hirose et al[25] for Swedish vowels. Miyawaki et al placed 5 electrodes from the anterior to the posterior portions of the genioglossus muscle, labeling them GG1 to GG5 anteroposteriorly. They found 2 distinct patterns of electromyographic activity for the Japanese vowel groups. Higher activity was noted on posteriorly located electrodes during the production of /i/, /e/, and /u/, so-called high front vowels. Production of /a/ and /o/ sounds showed lower activity. They suggested the presence of functionally distinct components of the genioglossus muscle with separate neuromuscular units ("motor unit territories").

The genioglossus muscle is known to be responsible for the maintenance of the mesopharyngeal airway when the body is in the supine position. Sauerland and Mitchell[21] called the genioglossus muscle a "safety muscle." The maintenance of tonic activity of the muscle by the stretch receptors seems to play a crucial role in prevention of posterior collapse of the tongue when the body is in the supine position. The cause of obstructive sleep apnea has been partially attributed to the position of the tongue.

In this preliminary study, we could not detect in the genioglossus muscle the functional units that had been presumed by Smith,[11] Miyawaki et al,[12] and others, but we confirmed its gradually successive structure. The average diameters of the muscle fibers gradually increased from the GGA to the GGP. The histochemical study showed that this gradual change was independent of the type of muscle fiber; ie, both types were changed. Hukunaga[26] reported that absolute muscle strength was in proportion to the diameter of the muscle. From this view, it is suggested that the absolute muscle strength of the genioglossus muscle gradually increases from the GGA to the GGP. Thus, the genioglossus muscle may show a gradual change anteroposteriorly, rather than having distinct functional units.

It has been reported that if one fiber type made up more than 55% of a muscle, that fiber type was significantly predominant.[27] In this histochemical study, the type II fibers of the GGA represented 68.7%, and it is thought that type II fibers were dominant in the GGA. On the other hand, the GGP had more type I fibers than the GGA, and there was no dominant fiber type. In general, it has been reported that type I

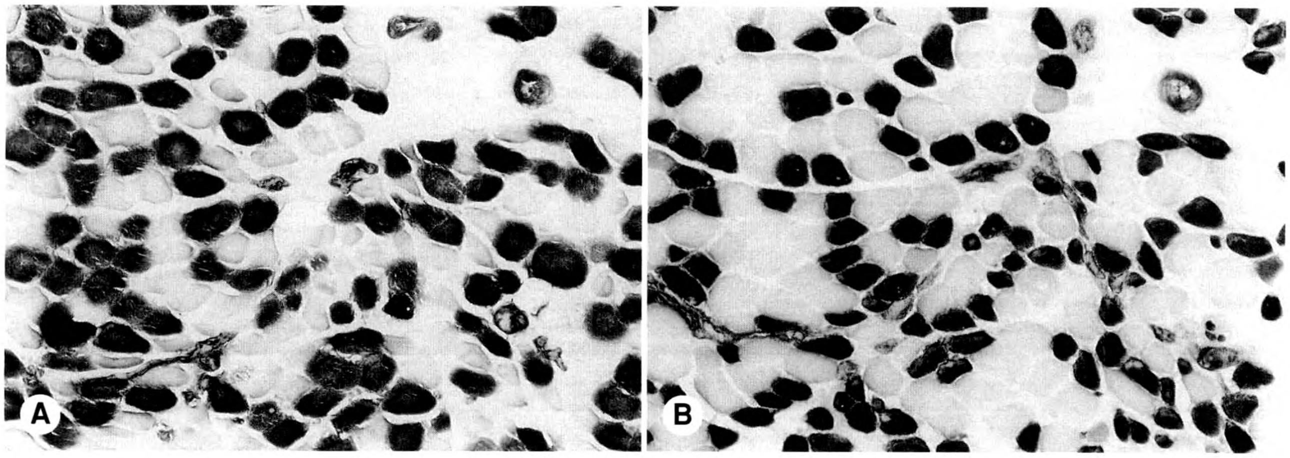

Fig 5. GGP stained by myosin adenosine triphosphatase reaction (original ×50). **A)** At pH 4.2. **B)** At pH 10.5.

TABLE 2. FREQUENCY AND SIZE OF TYPE I AND TYPE II FIBERS IN GENIOGLOSSUS MUSCLE

	Frequency (%) of Fibers		*Fiber Diameter (μm)*		
Portion	*Type I*	*Type II*	*Type I*	*Type II*	
			NS		
Anterior	31.3	68.7	24.5 ± 5.2	21.0 ± 6.5	
			*		*
Posterior	51	49	29.6 ± 6.5	34.6 ± 9.0	
			*		

Fiber types were identified with adenosine triphosphatase reaction at pH 10.5.

NS — not significant.

*p < .05.

fibers are responsible for slow contraction, being more adapted to tonic activity. Type II fibers would be responsible for contraction during voluntary movements corresponding to a phasic function.[28-30] Thus, it is suggested that the GGA is more suitable for phasic movement, and the GGP is relatively suitable for tonic activity. From this preliminary study, the GGP appears to have greater absolute muscle strength than the GGA and to be more suitable for tonic movement. Concerning the upper airway, it can be suggested that the GGP might contribute to the maintenance of the mesopharyngeal airway. The GGA may have more rapid and phasic movements. It can be suggested that the GGA might contribute to consonant production and some fine movements, for example, the click sound /tut/, and so on. Hellstrand[31] reported type II fibers at 74.6% and saw no clustering of fibers of any group, only irregular scattering, in the cat's genioglossus muscle. Anatomic differences between cat and human genioglossus muscles might be the result of differences in posture (quadrupedal versus bipedal) and related to speech function, unique to humans. It is thought from these results that the human genioglossus muscle may be able to manage its activity and degree of contraction successively with respect to its portions, so that the tongue changes in a more variable pattern morphologically than was assumed by the idea of the genioglossus muscle as separated into functional units. Further anatomic study may be warranted to elucidate the neuromotor control systems of the tongue.

ACKNOWLEDGMENT — We thank Associate Professor Kumiko Tanuma, Department of Anatomy, Nippon Medical School, for helpful advice and expert technical support.

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RHINOMANOMETRIC AND OLFACTOMETRIC VARIATIONS THROUGHOUT THE MENSTRUAL CYCLE

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A prospective study was performed to evaluate the rhinomanometric values and the changes in the olfactory threshold that occur in women throughout the menstrual cycle. The subjects were 60 healthy volunteer premenopausal women 18 to 40 years of age. We performed rhinomanometric and olfactometric measurements during the follicular, periovular, and luteal phases of the menstrual cycle. Rhinomanometry showed a higher (not statistically significant) airflow during the periovular phase than during the follicular and luteal phases. Olfactometry showed a higher sensitivity during the follicular phase ($p < .05$) and the periovular phase ($p < .001$) than during the luteal phase. We conclude that airflow and transnasal pressure during spontaneous respiration, as well as the olfactory threshold to odors, seem to depend on the variations of the ovarian steroids that occur during the menstrual cycle.

KEY WORDS — menstrual cycle, olfactometry, rhinomanometry.

INTRODUCTION

Chemical communication involves the transmission of a chemical signal from one organism to another. The emission of a signal may be intentional or may be a product of other activities. The important factor is that the emitted chemical should have informational value for another organism.[1]

The ability to detect chemical stimuli in the external environment is of fundamental importance to all animals. Chemoreception is used to locate and test food, to initiate escape from potentially harmful compounds and predators, to recognize mates and co-specifics, and to maintain a nutritional balance. For reasons not yet completely understood, women exhibit greater sensitivity to some odors than do men.[2,3] However, recent data suggest that there are no significant differences between men and women on the odor identifications test,[4] and that odor identification could depend on linguistic capabilities, familiarity with odorants, or odor memory.[5] On one level, the female advantage in humans appears to derive from hormonal factors or from other variables associated with changes in the hormonal status of the woman.[2] It seems that women are more sensitive than men to certain chemicals. For instance, in one study, sensitivity to exaltolide seemed to be strongly associated with hormonal status, since adult males and prepubertal females and males were all much less sensitive to the substance than were adult females.[6] Furthermore, sensitivity to exaltolide peaked just before the periovular phase of the menstrual cycle, and again during the luteal phase.[6] Studies on the correlation between olfactory sensitivity and a variety of physiological indicators, including estrone, estradiol, follicle-stimulating hormone, and luteinizing hormone, throughout the menstrual cycle demonstrated that peak sensitivity occurred during the second half of menses, at midcycle and mid-luteally.[7] Estrogens may be involved, since women taking oral contraceptives also demonstrated cyclical changes in olfactory sensitivity. Accessibility of odorants to the olfactory epithelium may vary with cyclical changes in the mucus layer, thus resulting in changes in olfactory sensitivity.[8]

In addition to vaginal odors,[9] other body odors also tended to be more pleasant during ovulation. The odor emitted from women around ovulation tended to stimulate and linger longer and at the same time produced a desire for more chemosensory stimulation.[10] This suggests that ovulation may thus be advertised to the human mate. The combined effect of these variables may convey a message of increased female alertness and attention during ovulation. Results of studies did indeed find that women reached the highest rate of coitus around ovulation.[11] Olfactory clues may be the main transmitters of information of the reproductive status of the female spider monkey, which, like the human female, does not show any visual signs of this status: spider monkey males sniff the females frequently at all phases of the sexual cycle.[12] Biologically, significant odors probably influence reproductive processes in males, and perhaps the notion of

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concealed ovulation in humans needs rethinking.[13] Ovulation in human females may be concealed from most co-specifics but be revealed to their mates in subtle chemical ways.[10] It has been demonstrated that the components of olfactory-evoked potentials are influenced by the phase of the menstrual cycle. The results show that women perceive olfactory stimuli with a higher sensitivity during the periovular phase.[14]

Ovarian cyclicity is a sensitive indicator of the physiological status of the female. Some parameters of the ovarian cycle, such as length and regularity, can be influenced by exogenous factors, and the exact values of these parameters can be reliable criteria for defining a woman's level of health. Some potential effectors of ovarian cycle parameters have been identified in a number of mammalian species, including humans.[15-18] Apparently, ovarian cycle parameters can be drastically changed by chemical cues originating from both male and female co-specifics. The menstrual cycles of some women who worked very closely with androstenone were shown to be altered, and there are influences of androstenol and androsterone on the evaluation of the attractiveness of various men.[19] There are two sensitive periods of olfactory stimulation during the menstrual cycle: follicular and luteal. Olfactory stimulation during the former caused a shortening of the menstrual cycle, while stimulation during the luteal phase caused lengthening. Periovular olfactory stimulation was ineffective, or less effective than follicular or luteal phase stimulation.[20]

Threshold values are subject to large individual differences and can vary considerably. This variation may be due, in part, to the different methods used by various investigators. Smoking, gender, aging, and blindness can have a tremendous effect on the measured threshold.[2,6] Several new psychophysical tests have recently become available for testing the sense of smell in a clinical setting. These tests can be useful in differential diagnosis and in assessing the extent of chemosensory loss. These are tests of olfactory sensitivity that use squeezable polypropylene bottles containing pyridine[21] or butane.[22] Another test, which measures suprathreshold function, is the University of Pennsylvania Smell Identification Test (UPSIT). It consists of a series of microencapsulated odorants presented in a multiple-choice format in booklets that can be used in the clinic or mailed to patients.[23]

Therefore, from the results of previous studies, it seems that currently there is only fragmentary research being carried out on olfactory cyclicity in women. The contact between odorous substances and receptors could depend on the flow quantity and the transnasal pressure during respiration. In turn, the contact between substances and receptors could depend on the different degrees of edema of the nasal mucosa during the 3 phases of the menstrual cycle. Thus, rhinomanometry could be useful in evaluating the olfactory differences during the menstrual cycle, and the olfactory threshold could depend on rhinomanometric characteristics.

We believe that 1) olfaction can have an important and interesting role during intimate experiences of women, 2) the success of sexual relations can depend on the olfactory canal, and that 3) any endogenous or iatrogenic hormonal modification can influence physiological and functional mechanisms.

Consequently, we wanted to study in what way the airflow and the olfactory threshold changed during the different phases of the menstrual cycle, in premenopausal women, so as to establish the baseline conditions for our studies on olfactory modifications caused by hormonal contraception or by hormone replacement therapy in postmenopausal women.

MATERIALS AND METHODS

The sample consisted of 64 women between 18 and 40 years of age, without known olfactory disorders. Subjects with tobacco or recreational drug use were excluded from the study. Moreover, the women enrolled in the study did not have any dysendocrinism, metabolic disease, or neoplastic disease. All women had the menstrual cycle with ovulation. To define the ovulation capacity of the cycle, we used sonography and determined the levels of serum progesterone during the luteal phase (21st to 25th days) of the cycle, the values of which were more than 18 IU/mL. A screening using endoscopy, rhinomanometry, and olfactometry was carried out. Endoscopy was used because it can aid in the differential diagnosis of olfactory dysfunctions. We used a Pentax FNL 10S (Pentax, Asahi Optical, Osaka, Japan) with a television camera and an SWHS AG 4700 VHS Panasonic recorder (Matsushita Electric Industrial Co, Osaka). This permits an unobstructed view of the nasal cavity and the rhinopharynx without a local anesthetic and allows recording of the images obtained. Rhinomanometry is defined as the simultaneous measurement of nasal airflow resistance (R) before and after nasal decongestion during spontaneous respiration. This resistance can be expressed by the formula $R = P/V$, where P is the transnasal pressure and V is the volume of airflow. We used a Rhinospir 164 (Sibelmed, Spain) for this test. To test the olfactory threshold, we used the Fortunato-Niccolini olfactometer (Catania, Italy) with 6 different odors. To evaluate the sensitivity differences that occur during the menstrual cycle, we carried out rhinomanometric and olfactometric surveys during the fol-

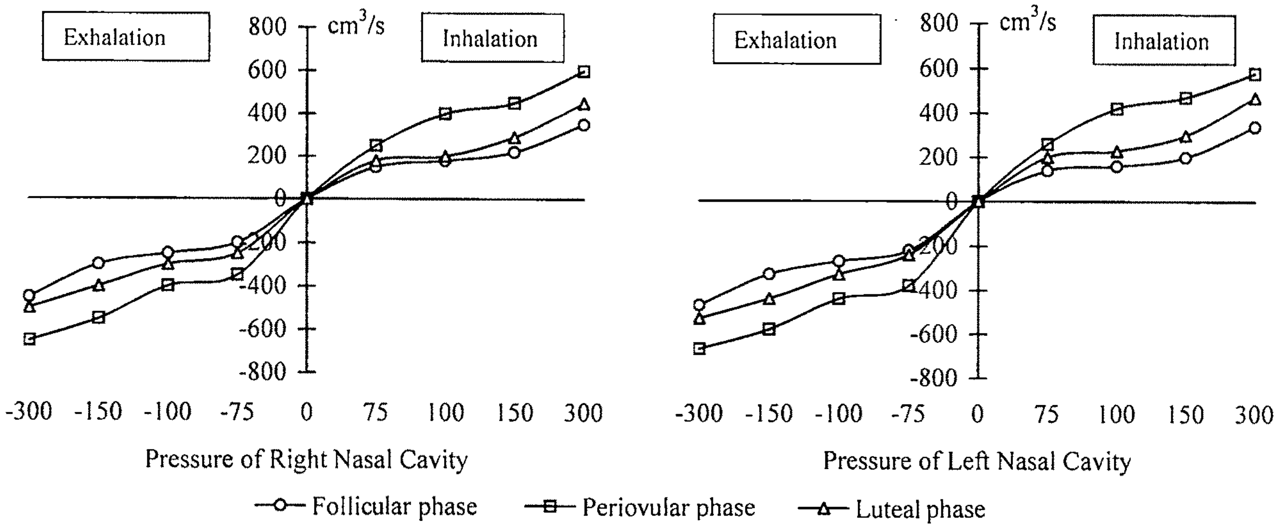

Fig 1. Rhinomanometric surveys of right and left nasal cavities of women during menstrual cycle. Women showed changes that were not statistically significant ($p > .05$) on rhinomanometric values between phases of menstrual cycle. Data are mean ± SD.

licular phase (days 5 to 8), the periovular phase (days 13 to 16), and the luteal phase (days 18 to 23). Rhinomanometry was performed on each woman during both inhalation and exhalation, with transnasal pressure ranging between 300 and −300 mm Hg, and with airflow ranging between 800 and −800 cm^3/s.

The substances used to test olfactory acuity with the Fortunato-Niccolini olfactometer are among the most suitable for a complete investigation: anise and musk ketone, which have an exclusive olfactory component; clove and pyridine, which, apart from the olfactory effect, add to the stimulation of taste and trigeminal terminations; citral, with an exclusive olfactory and trigeminal effect; and ammonia, with a prevalent trigeminal component.[24-27] Each substance was used in the liquid state. Anise, musk ketone, citral, and clove were pure essences; pyridine was a pure liquid substance; ammonia was a liquid substance diluted to 22% in distilled water (Farmacia, Milan, Italy). We considered the olfactory coefficient of each substance, which is the quantity of odorous air sufficient to reach the level of identification over 5 seconds: 2 cm^3 for anise, musk ketone, citral, and clove; 0.5 cm^3 for ammonia; and 1 cm^3 for pyridine. Usually, a subject who does not perceive any substance in the range of its specific olfactory coefficient over 5 seconds is defined as affected by hyposmia or anosmia. The Fortunato-Niccolini olfactometer has 6 glass bottles of 500 cm^3. Each of the 6 bottles contained 30 cm^3 of a single substance. Each bottle was hermetically sealed by a rubber cap through which 2 tubes were passed: one connected to a specially graduated syringe, the other to a connector leading to the nostrils. The graduated syringe was actuated by a controlled pressure system. The pressure was created so as to blow an increasing quantity of the specific substance into each bottle, with an increase of 0.2 cm^3 per test, up to its olfactory coefficient. The olfactometric test was made on all patients sitting in front of the terminal fork, which conducted the odors simultaneously to both the right and the left nasal vestibules. In turn, the valve of the tube connected to the nostrils was opened to allow the passage of air saturated with that odor. Each odor was delivered to the nasal mucosa during normal inspiration. Bearing in mind the different degrees of edema of the nasal mucosa during the 3 phases of the menstrual cycle, we repeated the test for each of the odorous substances until the patient no longer perceived the odor, by gradually stopping the pressure syringe system. The perception threshold was defined as the smallest volume of air able to make the subject perceive the odor.

Statistical analyses were carried out by analyses of variance for the planned comparisons phase and odor. The Spearman test was used for statistical analysis. The differences were considered statistically significant when the p value was less than .05.

RESULTS

After rhinoscopy, 3 subjects affected by polyps associated with allergic rhinitis, and 1 affected by a deviated septum, were excluded from this study. Therefore, the sample consisted of 60 women between 18 and 40 years of age. Rhinomanometry excluded the presence of physiological anatomic alterations that would impede the contact of odorous substances with their receptors, such as atrophic lesions of the nasal mucosa caused by toxic agents (eg, tobacco), drugs (eg, vasoconstrictors), or commercial agents (eg, paints, solvents, perfumes).

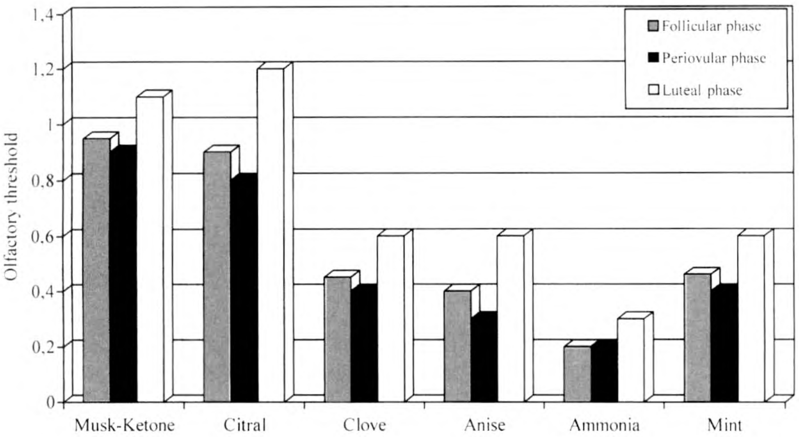

Fig 2. Olfactometric thresholds of women during menstrual cycle. Women showed higher olfactometric threshold sensitivity during both follicular ($p < .05$) and periovular phases ($p < .001$) than during luteal phase of menstrual cycle. Data are mean ± SD.

Figure 1 shows rhinometric values obtained during each phase of the menstrual cycle. Each subject had a higher (not statistically significant) airflow during the periovular phase than during the follicular phase ($p = .86$) and the luteal phase ($p = .82$). For each menstrual phase, the mean transnasal pressure, with respect to the airflow value during inhalation and the exhalation time, of the right nasal cavity was similar to that of the left nasal cavity (not significantly different).

Figure 2 shows the olfactometric thresholds of each phase of the menstrual cycle. Olfactometric threshold data indicated a higher sensitivity 1) during both the follicular phase ($p < .05$) and the periovular phase ($p < .001$) than during the luteal phase, and 2) during the periovular phase than during the follicular phase ($p < .05$), for the substances with an exclusive olfactory component (musk ketone and anise) and for those with both olfactory and trigeminal effects (clove, mint, and citral). For ammonia, which has a prevalent trigeminal effect, the olfactory threshold during the follicular phase was similar to that of the periovular phase (not significantly different), and no statistically significant differences were observed between the follicular phase or the periovular phase and the luteal phase.

CONCLUSIONS

The airflow and transnasal pressure that occur during spontaneous respiration, as well as the olfactory threshold to odors, seem to depend on qualitative and quantitative changes in ovarian hormones that occur during the menstrual ovulatory cycle.

We observed that the highest degree of olfactory sensitivity coincides with ovulation. Other authors did not find any changes in olfactory sensitivity during the menstrual cycle, probably because the exact time of ovulation was not determined.[21,22] Moreover, there may be 2 peaks of olfactory sensitivity, occurring during the mid-cycle and the mid-luteal phases.[23] We confirmed, by sonography and by measuring the serum levels of progesterone, that each woman was in the periovular phase of the menstrual cycle at the time we performed the rhinomanometric and olfactometric tests.

Although our data confirm the existence of olfactory variations during the menstrual cycle, we think that further studies are needed to investigate in what ways olfaction is affected by treatment with steroids such as oral contraceptives used by premenopausal women and hormone replacement therapy used by postmenopausal women.

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PHENYTOIN-INDUCED LINGUAL TONSIL HYPERPLASIA CAUSING LARYNGEAL OBSTRUCTION

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The effects of phenytoin sodium on lymphoid tissue have been known for some time. However, its effect on the lymphoid tissue of Waldeyer's ring is unreported in the otolaryngology literature. We present the case of a 78-year-old woman who was treated with phenytoin for generalized tonic-clonic seizures for 4 months and who subsequently developed lingual hyperplasia that caused laryngeal obstruction eventually requiring a tracheotomy. Of note, no history of airway compromise or difficulty with intubation from a prior operation 20 years ago was reported; these findings suggest that the lingual tonsillar hyperplasia was a consequence of phenytoin therapy and not a preexisting condition. The findings of histopathologic evaluation were consistent with lymphoid hyperplasia without evidence of malignancy. We also report our operative management and follow-up of this rare condition.

KEY WORDS — Dilantin, laryngeal obstruction, lymphoid hyperplasia, phenytoin, tonsillectomy.

INTRODUCTION

The causes of airway obstruction can range from the benign to the malignant. As experts of the airway, it behooves otolaryngologists to be aware of all the possibilities, whether from an exogenous or an endogenous source. We have had the opportunity to experience and report a rare but interesting source of upper airway obstruction, namely, that due to hyperplasia of the lingual tonsils as a result of phenytoin sodium (Dilantin) treatment.

Two rare but well-documented complications of phenytoin's use as an antiepileptic agent are lymphoproliferation and lymphadenopathy.[1-6] These lymphoproliferative disorders usually fall into 1 of 4 distinct categories that vary from benign lymphoid hyperplasia to true malignant lymphoma. The cases reported in the literature, however, have all occurred outside the larynx and pharynx.[1-8] We believe that this case represents the first report of phenytoin-induced lingual tonsillar hyperplasia.

CASE REPORT

A 78-year-old woman had a medical history significant for cardiovascular disease and non–insulin-dependent diabetes and a surgical history significant for a hysterectomy performed 20 years earlier. She presented to the neurosurgical service of Jacobi Medical Center in March of 1998 after experiencing right focal motor seizures and receptive aphasia. She was placed on a maintenance dose of 300 mg Dilantin at that time. Subsequent diagnostic evaluation revealed a large left parasagittal mass compressing the left frontal and parietal lobes consistent with a meningioma. In an effort to suppress seizure activity, the dosage of Dilantin was maintained for approximately 3 months before she was scheduled to undergo craniotomy and resection of the meningioma.

The preanesthesia assessment was significant for maxillary and mandibular gingival hyperplasia, the finding of a class 1 oral cavity, and poor dentition. Specifically, no stridor or shortness of breath was noted. On the day of surgery, the patient underwent intravenous induction of anesthesia in preparation for intubation. However, the anesthesiologists had to make multiple attempts at intubation, finally having success with a No. 3 Miller blade and a guidewire. It was noted that the patient had abnormal airway anatomy and that it was difficult to visualize the epiglottis or vocal cords because of obstructing hypopharyngeal tissue. The planned neurosurgical operation proceeded without incident, and the patient was extubated and sent to the recovery room in stable condition. While in the recovery room, however, the patient suffered a tonic-clonic seizure and became apneic. As reintubation was problematic because of the aforementioned obstructing mass, an emergent cricothyroidotomy was performed. The otolaryngology service was immediately consulted to rule out any hypopharyngeal, laryngeal, or tracheal trauma. Direct laryngoscopy confirmed the presence of redundant and obstructing tissue in the hypopharynx, and a preliminary diagnosis of laryngeal papillomatosis

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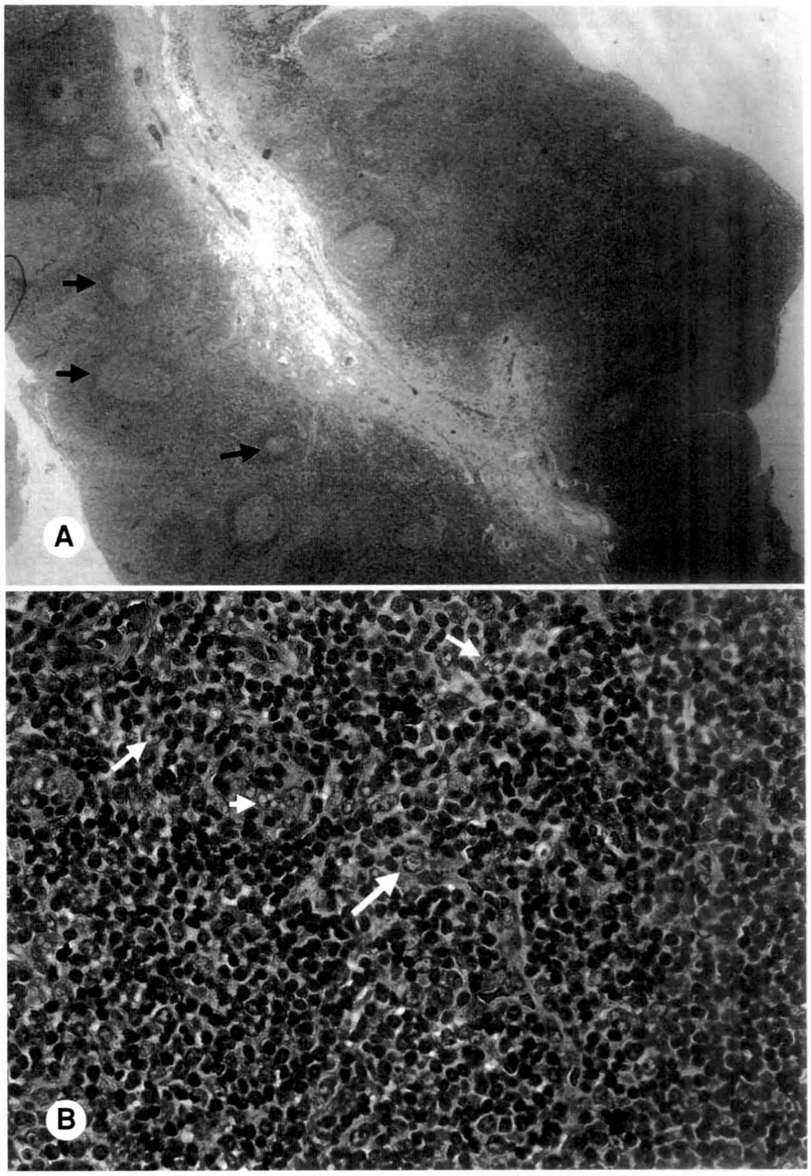

Hematoxylin and eosin–stained section through patient's lingual tonsil specimen. **A**) Original ×2.5. Shows variation in size of germinal centers (arrows). **B**) Original ×10. Shows uniform lymphocyte population and presence of normal number of histiocytes (arrows).

was made. On the second postoperative day, the otolaryngology service converted the cricothyroidotomy to a tracheotomy.

Subsequently, the patient improved neurologically but continued to require a tracheotomy for upper airway obstruction. On postoperative day 5, she was aphonic and dyspneic when her fenestrated, cuffless tracheotomy tube was occluded. A follow-up otolaryngological examination on postoperative day 20 revealed a weak and raspy voice, an oral cavity with significant gingival hyperplasia, and hyperplastic lymphoid-appearing tissue originating from the base of the tongue and completely obstructing the view of her epiglottis and vocal cords. At this point, the diagnosis of lingual tonsillar hyperplasia was made, and the patient consented to direct laryngoscopy and excision of the lingual tonsils.

The patient was taken to the operating room for direct laryngoscopy and lingual tonsillectomy. Inspection of the tongue base revealed large, redundant, and overlapping lingual tonsils that completely obscured the epiglottis and vocal cords. The epiglottis and false and true vocal cords appeared normal. The tonsillar hyperplasia was limited solely to the lingual component of Waldeyer's ring. The hyperplastic tissue was excised with electrocautery without complication. Tissue samples were submitted to pathology, which revealed lymphoid hyperplasia with intact architecture grossly resembling a low-grade lymphoma. However, no focal necrosis or granulomas were identified, and hematoxylin and eosin staining of sections through the specimen demonstrated 1) the presence of an average number of histiocytes in the lymphoid population, 2) variation in the size of germinal centers, and 3) a uniform population of lymphocytes. All of these findings strongly suggested a benign process (see Figure). In light of the obvious histologic findings, no specific immunohistochemical staining was performed.

On postoperative day 2, the patient was able to tolerate occlusion of her tracheotomy tube. By postoperative day 5, the patient was successfully decannulated and discharged from the hospital. At a 3-week follow-up appointment, the patient expressed no respiratory complaints and exhibited a clear, strong voice. Her tracheotomy site was completely closed. She continued to take Dilantin as of December 2000.

DISCUSSION

The effects of hydantoins as a drug class (and phenytoin in particular) on the lymphoid tissue were first noted shortly after their introduction as anticonvulsive agents in the late 1930s.[9] The most extensive review of this phenomenon was performed in 1959 by Saltzstein and Ackerman,[4] who reviewed 75 cases of hydantoin-induced lymphadenopathy. As a result of their work and the reports of others, a clear association between phenytoin use and lymphoproliferative disorders, ranging from benign hyperplasia to malignant lymphoma, emerged.[3]

In 1968, Gams et al[10] categorized this entity into 4 major groups — hyperplasia, pseudolymphoma, pseudo-pseudolymphoma, and malignant lymphoma — collectively known as the pseudolymphoma syndrome. In the hyperplasia group, patients present with lymphadenopathy and often, but not always, have accompanying skin rash, fever, and hepatosplenomegaly. Histologically, the architecture of the biopsy specimen is intact. In the pseudolymphoma group, the clinical presentation is similar to that of the hyperplasia group, but nodal biopsy specimens reveal disruption of normal architecture, as well as pleomorphic cell proliferation. Of particular note is the fact that discontinuation of the use of phenytoin will result in a marked resolution of signs and symptoms in these first 2 groups. The pseudo-pseudolymphoma group bears clinical and histologic similarities to the pseudolymphoma group, except that discontinuation of phenytoin will not result in a complete resolution of the disease, which, after a latency period, recurs as a true malignant lymphoma. Finally, the malignant lymphoma group demonstrates clinical and histologic features of malignant disease that do not regress after drug discontinuation.

The case presented has many features consistent with the hyperplasia subgroup of Gams et al[10] of the phenytoin-induced pseudolymphoma syndrome. The patient was treated with a common phenytoin preparation (Dilantin) for 3 months and exhibited gingival hyperplasia, which is the hallmark of long-standing phenytoin use. Most reported cases of pseudolymphoma syndrome occur within 3 months of the onset of therapy.[1] Although the patient did not develop a rash, she did experience several episodes of fever, shaking, and chills during her treatment period. The unusual feature of this particular case is the extranodal presentation of lymphoproliferation, given that the patient demonstrated lingual tonsillar hyperplasia instead of the lymphadenopathy seen in most cases of pseudolymphoma syndrome. Interestingly, the hyperplasia of her lingual tonsils would most likely have gone undetected had it not been an obstruction to the airway during intubation, eventually necessitating a tracheotomy. While it is impossible to be absolutely sure that her lingual tonsils were not hyperplastic before Dilantin use, the fact that she underwent general endotracheal intubation 20 years earlier for a hysterectomy without any noted complications or difficulties suggests that the finding was not a long-standing anatomic aberration.

While Waldeyer's ring may be a novel location for the phenytoin-induced pseudolymphoma syndrome, it is not an unusual location for malignant lymphomas. In fact, both undifferentiated and lymphoblastic lymphomas show a predilection for Waldeyer's ring.[11]

The histopathologic characteristics of our patient's tissue specimen also suggested that her case conformed to the hyperplasia subgroup of phenytoin-induced pseudolymphoma syndrome. The specimen revealed lymphoid hyperplasia with intact architecture resembling a low-grade lymphoma. No focal necrosis, granulomas, or Reed-Sternberg cells were identified. In addition, the uniform shape and size of the lymphocytes, the presence of an average population of histiocytes, and the lack of angulated and irregular lymphocyte nuclei were consistent with a benign hyperplastic process. No specific immunohistochemical studies were performed, given the classic signs of benign disease.

The treatment for symptoms caused by the pseudolymphoma syndrome is ultimately discontinuation of the offending hydantoin. As mentioned above, this results in the regression of lymphadenopathy and other associated symptoms in the hyperplasia and pseudolymphoma subgroups. However, since our patient presented with lymphoproliferation obstructing the airway and complicating tracheotomy decannulation, it behooved us to operatively debulk the lingual tonsils. Immediate improvement in airway clearance was noted, as the patient was able to tolerate occlusion of her tracheotomy tube postoperatively.

Although the pseudolymphoma syndrome is extensively discussed in the literature, it is our belief that its presentation as lingual hyperplasia is unusual and heretofore not reported in the otolaryngology literature. It represents yet another disease process that can obstruct the airway and thus warrants consideration in patients on anticonvulsive medication who present with symptoms of airway obstruction.

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IMAGING CASE STUDY OF THE MONTH

SPONTANEOUS DISSECTION OF THE INTERNAL CAROTID ARTERY IN ITS EXTRACRANIAL PORTION, REVEALED BY A HYPOGLOSSAL PARALYSIS: REPORT OF FOUR CASES

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PARIS, FRANCE

INTRODUCTION

Dissections of the arteries supplying blood to the brain represent 2% to 4% of the cerebrovascular diseases in the general population. In young adults, this percentage rises to 20%.[1,2] The internal carotid artery (ICA) is the most commonly involved.[3] In most cases, it is revealed by an acute apoplectic stroke and is assumed by the neurologist. Rarely, it can manifest itself by the attack of 1 or more of the last 4 cranial nerves (glossopharyngeal [9th], vagus [10th], spinal accessory [11th], hypoglossal [12th]) in the carotid space, without any associated cerebrovascular disease. The hypoglossal nerve is the most often involved in this case, and the opinion of the otolaryngologist may then be required.

We report 4 cases of dissection of the ICA in its extracranial portion that were revealed by a hypoglossal paralysis. We describe the cases and discuss the methods for diagnosis and treatment of this rare syndrome.

CASE REPORTS

We report 4 cases of subadventitial dissection of the extracranial ICA. At first, no sign of a central-type neurologic stroke was apparent. As first described by other authors,[1] the dissection started 2 cm above the carotid bulb and did not involve the intrapetrous portion of the ICA.

Case 1. A 65-year-old man with no significant medical history had first consulted an ophthalmologist because of a sensation of edema in the right superior eyelid that had started 24 hours earlier. He also complained of a 1-month history of frontal headache. An allergy was suspected, but a few days later, the patient complained of right-sided facial paresthesia, and 2 weeks later, he complained of difficulty in swallowing. The patient was referred to our department at this time. The clinical examination revealed a right-sided Horner-Bernard syndrome and a typical right-sided hypoglossal paralysis. The findings of fiberoptic endoscopy of the pharynx and of the larynx were normal. Cervical palpation revealed no mass able to compress the hypoglossal nerve and/or the cervical sympathetic nerve. The diagnosis of a spontaneous dissection of the ICA was made and was confirmed on magnetic resonance imaging (MRI) that was performed the same day. On T1-weighted images, there was a crescent-type wall hematoma in the last upper 2 cm of the extracranial portion of the ICA. This hematoma became more evident on T1-weighted images with fat suppression. After 8 weeks of anticoagulant treatment (heparin calcium followed by coumarin anticoagulant), the clinical recovery was complete.

Case 2. A 54-year-old man, a smoker, with excess weight and arterial hypertension that was well treated by a beta-blocker, complained of a chronic diffuse headache and presented in 1994 to the department of cardiology because of difficulty in articulating speech that had appeared suddenly the same day, after a headache of moderate intensity. He presented dysarthria, open rhinolalia, dysphonia, and left-sided deviation of the tongue. The patient was referred to a neurologist and then to an otolaryngologist. Clinical examination revealed an incomplete paralysis of the left 9th, 10th, and 12th cranial nerves. An MRI scan revealed a crescent-like hematoma 3 cm long in the wall of the left ICA, with high intensity on T1- and T2-weighted images, starting 1 cm above the ca-

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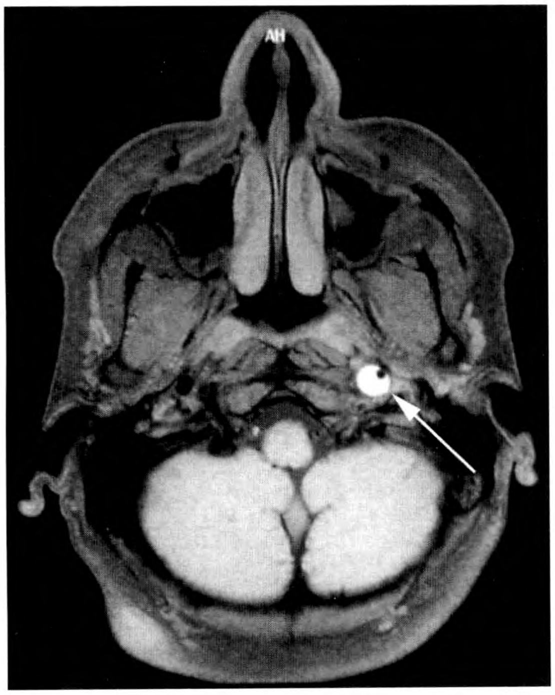

Fig 1. T1-weighted axial image with fat saturation, showing crescent-like dissection of left internal carotid artery in its cervical portion (arrow).

rotid fork. Use of magnetic resonance angiography showed a thinning of the arterial lumina all along this 3-cm area. An echo Doppler investigation of the cervical vessels yielded normal findings. After 4 weeks of anticoagulant treatment (heparin followed by coumarin), the clinical recovery was complete, and magnetic resonance angiography showed that the arterial flow had recovered, although an irregularity of the arterial walls of the left ICA persisted.

Case 3. A 54-year-old man (nonsmoker and nondrinker) had a left-sided temporal headache associated with a homolateral otalgia. There was neither an infectious nor a traumatic context. He presented to our department several days later because of difficulty in masticating. The clinical examination revealed a left hypoglossal paralysis. The neurologic findings were in other respects normal, as were the findings of cervical palpation (no lymph node that could compress the nerve) and nasal and pharyngeal fiberoptic endoscopy. The patient refused to be hospitalized in spite of the practitioner's insistence. Eight days later, he had a cerebrovascular accident that consisted of right-sided hemiplegia, aphasia, and paralysis of the left vagus, left glossopharyngeal, and left hypoglossal nerves. An echo Doppler examination of the cervical vessels revealed an occlusion of the left ICA. An MRI scan showed ischemic-type cerebrovascular disease in the left superficial sylvian territory. In addition, it showed a significant stenosis of the left ICA above its bulb, in the form of a 2-cm-long wall hematoma starting 1.5 cm above the carotid fork. This hematoma had a high signal intensity on T1-weighted images. Another MRI scan performed 1 month later showed a permeable left ICA with only a slight diminution of its caliber. At the 4-year follow-up visit, the patient had totally recovered the motoricity of his right leg, but he still had aphasia and paralysis of his right arm and had not returned to work.

Case 4. A 43-year-old man with no significant medical history (nonsmoker and nondrinker) had been complaining for 8 days of left-sided odynophagia, left-sided periorbital pain, and transitory headache, for which he had been treated with antibiotics and acetaminophen, when he complained of sudden difficulties in swallowing and articulating. He was sent to the hospital, where a clinical examination revealed a left isolated hypoglossal paralysis, with normal findings on cervical palpation. An MRI scan performed the same day showed a wall hematoma on the left ICA, extending from its origin to 1 cm below the skull base (Fig 1). After 3 weeks of medical treatment, he had not recovered his nervous function, and there was still no improvement at the 24-month follow-up.

DISCUSSION

Cases of dissection of the ICA in its cervical portion are rarely reported in the international literature. Moreover, the few cases reported tend to be in meta-analyses.

Symptomatology. Two types of symptoms can be observed. Central symptoms are due to cerebral ischemia. Local symptoms are due to the dissecting hematoma in the arterial wall. Extracranial dissection of the ICA attacks 1 or more of the cranial nerves without a cerebrovascular accident, in 6% of cases according to Hart and Easton,[3] and in 9% of cases according to Ast et al.[4]

Local symptoms are observed in all cases without a cerebrovascular accident and in most cases with a cerebrovascular accident. These local symptoms can be either isolated or combined. They are, in descending order of frequency, headaches, Horner-Bernard syndrome, pulsatile tinnitus (found in 15% of cases), and cranial nerve paralysis.

The initial symptom in 60% to 70% of cases, the headaches of ICA dissection have a changeable character.[1,3,5] They are most often localized to the same side as the dissected ICA. They are generally frontal and/or orbital, permanent, and of intermediate to high intensity, and last several days.[5,6] They can also sometimes be nontypical and resemble migraine or a facial cluster headache.[7] Cervical pain is noted in 25% of cases.

Horner-Bernard syndrome is observed in 20% to

58% of cases.[5,8] It is due to a stroke of the pericarotid sympathetic plexus or of the superior sympathetic node. The syndrome can be painful. D'Anglejan Chatillon et al[1] report 8 cases of dissection of the cervical ICA that had manifested themselves only with local symptoms. Horner-Bernard syndrome was observed in 7 of these 8 cases.[1]

The cranial nerve paralysis, either isolated or combined, concerns 1 or more of the last 4 cranial nerves. The hypoglossal nerve is the most often involved. Hart and Easton[3] report a 5% incidence of hypoglossal paralysis in 146 cases of dissection of the cervical ICA. Serre et al[9] concluded, after review of 32 cases of hypoglossal paralysis due to a dissection of the extracranial ICA reported in the international literature, that such a paralysis is never isolated, but is associated with Horner-Bernard syndrome, palsy of another cranial nerve, hemiparesis, or aphasia. However, some authors report cases of isolated hypoglossal paralysis.[10,11]

The association of headache with hypoglossal paralysis and Horner-Bernard syndrome is the most frequent and the most specific clinical manifestation of a dissection of the cervical ICA. Less frequently, ICA dissection can be associated with a palsy of 1 or more of the 9th, 10th and 11th cranial nerves. However, such palsies, without central symptoms, can occur in other diseases for which treatment is less urgent (tumors or metastases of the skull base).[12]

The central symptoms consist of cerebral ischemia, which we will not detail. These symptoms are present in 90% of cases, and they are the initial symptom in 30% of cases. When they appear secondarily, they can do so a few minutes to 15 days after the appearance of the local symptoms, as in our third case.

Pathology. The carotid dissection is due to bleeding in the arterial wall. There are 2 types of arterial dissections.[8] The first type is a subintimal hematoma, which most often causes stenosis and even occlusion of the artery. The risk of a secondary cerebrovascular event is important and is due either to the stenosis or occlusion or to a cerebral embolism. The second type, which is the most probable in our series, is a subadventitial hematoma, which can be separated from the true lumen of the vessel. This type of hematoma develops most often outside the artery and thus compresses the nervous elements that pass close to the ICA in the posterior lateral pharyngeal space (cervical sympathetic nerve, hypoglossal nerve, and, less often, 9th, 10th, and 11th nerves). Sometimes, the true lumen of the artery is normal. Intraparietal hematomas can create a partial stenosis that is well tolerated, as in our second and third cases. They rarely induce a complete occlusion.

The usual slight narrowing of the artery's true lumen explains the lower number of cerebrovascular events that occur when the hematoma is of the subadventitial type. Nevertheless, their prevention necessitates anticoagulant treatment. Subadventitial hematomas generally result in recovery. The lower rate of subadventitial dissections as compared to subintimal ones explains the rare occurrence of dissections of the extracranial ICA that manifest themselves with an isolated paralysis of cranial nerves.[11]

Etiopathology. In the majority of cases, the cause of this disease, which concerns especially young adults, is not found. Some risk factors are nonetheless well known. The most common are cervical traumas, especially in young adults,[13] and maybe repetitive small traumas, such as even neck extensions; fibromuscular dysplasia, which is found in 13% to 20% of cases[1,3,14]; arterial hypertension; and estrogen-progesterone treatments.

Imaging. Three techniques of radiologic imaging permit diagnosis of dissection of the ICA in its extracranial portion. The first, cervical and transcranial echo Doppler imaging, is relatively easy to perform and thus can be useful in an emergency.[15] Nevertheless, it necessitates a trained operator and seems not to be sufficient, because it will not permit diagnosis of a subadventitial dissecting aneurysm with a normal vascular flow.

The second technique is conventional angiography. Ast et al[4] report 68 cases, 91% having a cerebrovascular event and 9% having no central neurologic sign. All these patients underwent a carotid system arteriography. There was a stenosis in 58% of cases, an occlusion in 35% of cases, a dissecting aneurysm in 13% of cases, and no instance of a double channel.[4] In cases of intraparietal hematoma without arterial occlusion or stenosis, the conventional angiography findings can be normal, or the artery's diameter may be only slightly larger than that of the opposite artery. The 4 cervical arterial axes must be explored to search for a dissection of the vertebral arteries, which exists in 10% of cases.[6,7]

Magnetic resonance imaging is the third technique and is the radiologic examination of choice for this disease. In case of a paralysis of the cervical sympathetic nerve or of the last 4 cranial nerves, there is always a parietal hematoma, well visualized with MRI after a few days, when it is composed of methemoglobin. The details of this technique are well documented.[16-18] It is necessary to include cervical and encephalic T1- and T2-weighted images in axial

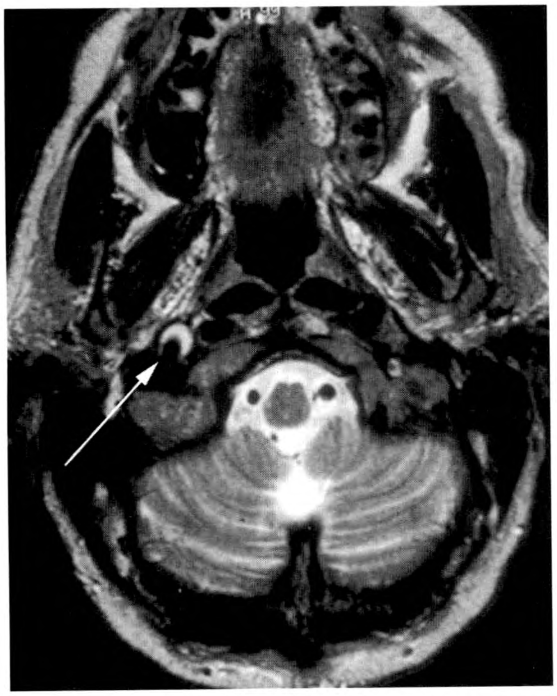

Fig 2. T2-weighted image in axial plane, showing crescent-like hematoma of right internal carotid artery wall (arrow). There is only slight diminution of arterial lumen.

planes. On the other hand, intravenous injection of gadolinium is not essential. On T2- and T1-weighted images in axial planes (Figs 2 and 3), an intraparietal hematoma is revealed by a circular or crescent-like high-intensity signal around a round, low-intensity signal that is due to the residual arterial flow. On T1-weighted images with fat suppression, the hematoma is well seen, appearing as an intense blush, compared to the perivascular tissues, which have an intermediate signal intensity (Fig 4). The location and the size of the hematoma are precisely defined.[16-18] The cerebral images show also whether there is associated cerebrovascular disease. The sensitivity of MRI depends on the size of the hematoma and in particular on its longitudinal extent. The machines of the latest generation (1.5T) have greater reliability, thanks to T1-weighted images with fat suppression and flow sequences.

Flow sequences show the permeability of the ICA and permit visualization in 3 dimensions of the intracranial and extracranial arteries.[19] The 4 cervical arterial axes must be explored, because other arteries, especially the vertebral arteries, are also involved in a dissection in 10% of cases.[6,7,20] Magnetic resonance angiography has a sensitivity of 95% and a specificity of 99%,[21] whereas T1-weighted images have a sensitivity of 84% and a specificity of 99%.[22]

Treatment and Prognosis. Surgery is not recommended for extracranial ICA dissections, because it is dangerous. According to most authors, the standard treatment consists of long-term systemic anticoagulation: intravenous injections of heparin are replaced by coumarin as soon as possible, and coumarin is given for at least several weeks (usually for 3 months). The effectiveness of this medical treatment has not yet been proven. Its goals are, first, to restore

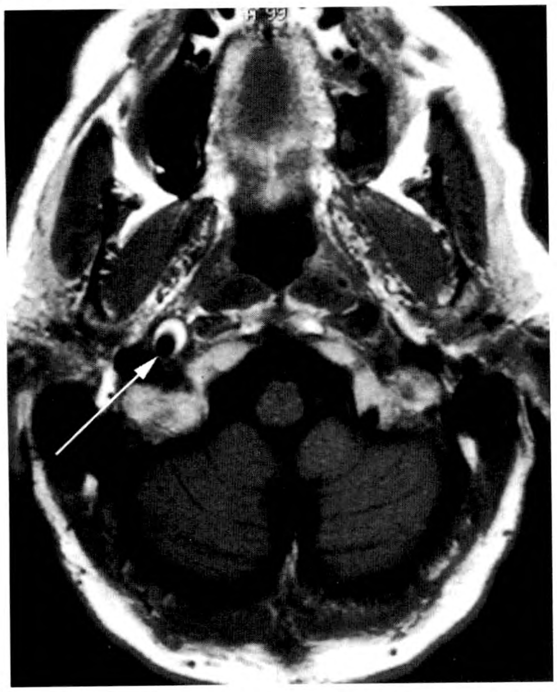

Fig 3. T1-weighted image of same case as in Fig 2. Wall hematoma has same aspect as T2-weighted image (arrow) — sign that hematoma is full of methemoglobin.

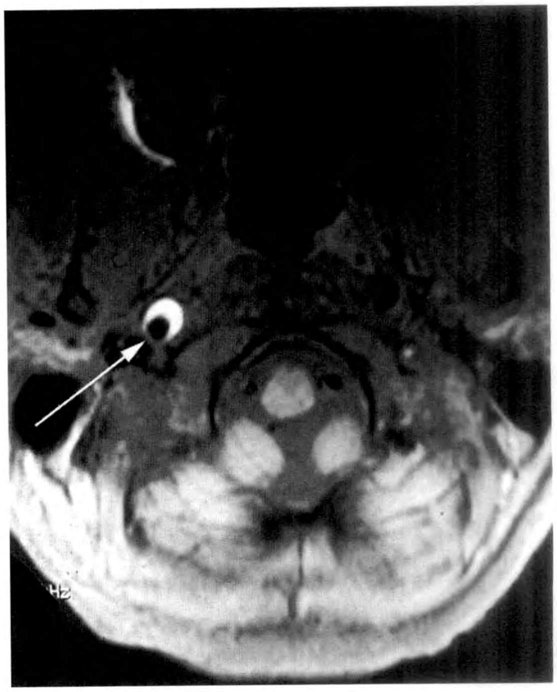

Fig 4. T1-weighted image of same case as in Figs 2 and 3, with saturation of fat. Carotid dissection appears clearly, with isolated high-intensity signal (arrow).

the permeability of the concerned artery and, second, to prevent an apoplectic stroke. In our third case, the natural evolution of the disease was a cerebrovascular accident that occurred 8 days after the hypoglossal paralysis. We think that heparin, given as soon as the hypoglossal paralysis appeared, could have prevented this negative outcome and its aftereffects.

The prognosis is generally favorable when there is no cerebrovascular accident. One can then observe a recovery of the arterial permeability and a recovery from the nerve palsy in almost 100% of cases. In case of a cerebrovascular accident, the prognosis depends on the possible aftereffects of the stroke. D'Anglejan Chatillon et al[1] report, concerning 62 patients treated with systemic anticoagulation, that 48% had complete recovery, 18% had slight aftereffects, and 5% died. Of their patients, 68% returned to their previous employment.[1]

CONCLUSION

Although spontaneous dissection of the ICA in its extracranial portion is an uncommon disease, usually treated in a department of medicine, it must be familiar to the otolaryngologist, who can diagnose the forms without central signs and especially those with hypoglossal paralysis, whether isolated or not. In the presence of such a paralysis, especially if it is associated with Horner-Bernard syndrome and if there is no compressive cervical lymph node, one must suspect a spontaneous dissection of the ICA in its extracranial portion and must urge a cervico-encephalic MRI scan, which is the examination of choice because of its reliability and relative safety. The treatment consists of long-term systemic anticoagulation with the goals of recovery of the dissected artery's permeability and prevention of a possible cerebrovascular event.

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LETTER TO THE EDITOR

To the Editor:

As the physician who contributed the data from Provident Hospital and was the primary author on the data used from Cook County Hospital,[1] I was concerned for several reasons when I read the article by Lim et al (Lim JW, Friedman M, Tanyeri H, Lazar A, Caldarelli DD. Experience with percutaneous dilational tracheostomy. Ann Otol Rhinol Laryngol 2000;109:791-6).

First, I question whether there is a reasonable scientific comparison between two procedures when patients are randomized to one procedure and then withdrawn from that group before undergoing the procedure. Thus, can groups 1 and 3 truly be comparable?

Second, this was not a prospective, randomized study, and there is no analysis to show that the groups are equal. Therefore, how can one know whether there is a true difference in the complication rates (which I am not sure is possible even if the groups are equivalent, as will be explained below)?

Third, group 3 did not consist of 211 consecutive patients from Cook County and Provident hospitals. There was one group of 100 patients, who were previously reported on,[1] who underwent percutaneous dilational tracheostomy (PDT) between 1988 and 1991. The second group had 156 PDTs performed at Provident from 1993 to 1998. Therefore, these groups were not continuous.

Fourth, I do not think that it is appropriate to include in this paper the 100 patients who were already reported on from Cook County Hospital. If they are included, why not include the 27 percutaneous dilational tracheostomy (PDT) patients from the randomized controlled trial from Cook County[2] that was performed after the 100 operations reported in this paper? More appropriately, these previously reported cases should not be included. Eliminating these patients from group 3 would significantly decrease the group 3 complication rates, eliminating 8 intraoperative and 10 postoperative complications, and probably alter the statistical significance.

Fifth, and most important, I do not understand why the authors disqualified 39 patients in group 3 because of abnormal anatomy, as they were not given any data regarding the patients' anatomy. However, they included all of the complications that I gave them for the 156 patients from Provident, as well as the previously reported complications from the 100 patients from Cook County. So, which 39 patients were excluded? If all of the patients with complications are included (and I do not think they should be, for the reasons given above), then the denominator would be 256 patients. This would lower the group 3 intraoperative complication rate to 3% and might affect the statistical significance.

Finally, I would like to know how the information was obtained that 19 patients in group 3 had bleeding complications. Only 3 patients from Provident and 5 patients from Cook County (who should not be included) had bleeding complications.

For all of these reasons, I have grave concerns regarding the data presented in the paper and, by extension, the conclusion that there are lower perioperative morbidity and mortality rates when a surgeon performs PDT than when a critical care specialist performs PDT. Many PDTs have been performed by critical care physicians who have reported low morbidity and mortality rates. The only way to truly answer the question the authors of this article have tried to answer, but not succeeded in answering, would be to perform a prospective randomized comparison of PDTs performed by a surgeon versus PDTs performed by a critical care physician.

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BOOK REVIEWS

Ménière's Disease

Jeffrey P. Harris, editor. Hard cover, illustrated, 418 pages, 1999. Kugler Publications, The Hague, the Netherlands, $183.

Purpose: Update.

Contents: A collection of offerings on every aspect of Ménière's disease by authorities who have reviewed current information regarding the mechanisms and preferred treatment of this disorder. Forty-one chapters make up the sections on pathogenesis, pathophysiology, diagnosis and clinical aspects, and treatment.

Attributes and Limitations: In 1842, Marie J. P. Flourens destroyed, individually, the semicircular canals and described the manifestation of the inner ear problem. Approximately 50 years later, Prosper Ménière knew that complete hearing loss had occurred when Flourens destroyed the semicircular canals, and drawing from approximately 20 years of clinical experience, he showed that vertigo was of labyrinthine origin. The pathophysiology of Ménière's disease emerged in the year 1939. Both Kyoshiro Yamakawa and Charles Hallpike (reporting with Sir Hugh Carnes) separately published the description of the histopathology of the inner ear in Ménière's disease. The cause of Ménière's disease is unequivocally unknown. Probably, Ménière's disease is not one disorder, but rather a group of different disorders. The book reviews a number of different perspectives on the present knowledge of Ménière's disease (or syndrome) and applicable treatments such as dexamethasone perfusion and the endolymphatic shunt. Garnered from the book are other typical topics of Ménière's disease, such as the following. 1) Hydrops does not provide a complete explanation for the physiological abnormalities associated with Ménière's disease. There are certain conditions, such as Mondini dysplasia and Klippel-Feil syndrome, that pathologically demonstrate endolymphatic hydrops yet do not have the associated triad of symptoms characteristic of Ménière's disease. 2) In most instances, there are relationships between hydrops and auditory and vestibular abnormalities. Seemingly, diuretics relieve some of the symptoms of hydrops. 3) The mechanism by which an acute Ménière's attack occurs still remains obscure, because it is not easy to conceive how a biochemical alteration occurs in such a severe manner. 4) In the classic work of Kimura and Schuknecht, occlusion of the endolymphatic duct produces symptoms of endolymphatic hydrops in a specific animal model, the chinchilla. Although the study still remains a classic, it is shown that such end results did not occur in other animal models, ie, the monkey, nor, probably, humans. The book provides a number of other important perspectives on the present knowledge of Ménière's disease. It is to be considered a review.

Applicability: Students, clinicians, researchers, and anyone interested in the relatively common condition of Ménière's disease.

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Otolith Function and Disorders

Patrice Tran Ba Huy and Michel Toupet, editors. Hard cover, illustrated, indexed, 132 pages, 2001. Karger, New York, NY, $147.

Purpose: This book fills a gap between semicircular canal function and central disorders by demonstrating the direct involvement of the utricle and saccule in pathology.

Contents: Presentations are taken from an international meeting devoted to "otolith function and disorders" held in Paris in January 2000. There are 9 chapters (manuscripts) that I would break down into groups of anatomic pathophysiology (4 chapters) and investigational instrumentation. Tests and/or equipment include eccentric or off-vertical rotary tests (a chair with adjustable tilt and lateral direction) and equipment for measurement of vestibular-evoked myogenic potentials (loud click-evoked potentials in the ipsilateral sternocleidomastoid muscle).

Discussion, Attributes, and Limitations: Using the information gleaned from their predecessors and from their own ingenious methods, which included selective nerve ablations, mechanical removal of portions of structures, and mechanical stimulation of the vestibular organ, scientists in the early 20th century laid bare the labyrinths of fish, guinea pigs, frogs, and pigeons and, later, cats, dogs, and monkeys, in an effort to learn more about the neurophysiology of the vestibular system. John Tait and William J. McNally confirmed the work of Flourens (100 years later) by showing that frogs failed to adjust their posture during movement in the plane of the destroyed canal. Other revelations in otolithic function were brought forth by Mario Camis, Rudolf Magnus, Adrian de Kleyn, Samuel Maxwell, and others. Their experiments demonstrated anatomic differences between *Homo sapiens* and lower forms of life, including erect gait, position of eyeballs, voluntary sitting, and the inhibiting influence of the central nervous system. It was not until 1936, with Tumarkin's report, that a clinical finding was perceived.

It is true that for the past 50 years, peripheral vestibular function and its related disorders have been attributed to the semicircular canal system. In such a respect, this is a timely book, in that it reintroduces revelations of otolithic function with 21st-century high-tech equipment. Peripheral and central vestibular syndromes that affect otolithic function (page 36) include posttraumatic otolith vertigo, Tumarkin's drop attacks, lymphatic fistula of the otolith type, vestibular atelectasis, otolith Tullio phenomenon, eighth nerve or labyrinth ocular tilt reaction, eighth nerve vestibular paroxysmia, labyrinth ocular tilt reaction, cortical vestibular epilepsy, and other rare central disorders. Diagnosis of each of these entities is still based on historical information. Appropriate testing is extensive, and the yield of results is still equivocal.

Applicability: Every otolaryngologist should read these excellent manuscripts to get acquainted with otolithic symptomatology for clinical diagnosis. Neurologists and physical therapists would also benefit from this knowledge, especially the latter, as the authors tend to emphasize therapy for relief of most of the symptoms, and such treatment is recommended rather than surgery.

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Emergencies of the Head and Neck

David W. Eisele and Shelly J. McQuone. Hard cover, illustrated, indexed, 447 pages, 2000. Mosby, St Louis, Mo, $99.

Purpose: It is hoped that this book will increase understanding of head and neck emergencies and enhance prompt recognition and successful management that will improve patient outcomes.

Contents: Included are emergencies that are of general concern (cardiopulmonary resuscitation, shock, endocrine, airway, foreign bodies in the aerodigestive tract) and those that are specific to the head and neck, including flap emergencies and dental, thoracic, intracranial, and ophthalmological emergencies. Each chapter, by and large, follows a certain format that includes the subject itself, presentation of the symptoms, evaluation, priorities, and outcomes.

Attributes and Limitations: This is a timely book. Patients are flooding into emergency departments because health care plans are required to pay emergency department bills if the patient is in great pain and believes he or she has a medical emergency (American Medical News 2001:44). Also, some patients are using the emergency department because they are having a difficult time getting an appointment to see their primary care physician. With more patients being "dumped" on the emergency room doctor, this book gives a more thorough understanding of head and neck problems and their prompt management.

The authors have created a scientific and academic book without losing any highly technical flavor. Tables that differentiate one entity from another add great strength to many chapters and are sorely needed in others. The photographic illustrations, although black-and-white, are well chosen and sharp, and are quite extraordinary.

Applicability: This book is a solid resource for the front line of specialty emergencies. Seasoned otolaryngologists can obtain new insights and up-to-date information on general specialty topics such as cardiopulmonary resuscitation, shock, and anesthetic emergencies.

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July 1, 2003

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Direct inquiries to:

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Children's Hospital & Regional Medical Center
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Seattle, WA 98105-0371
(206) 528-5075
fax: (206) 527-3955
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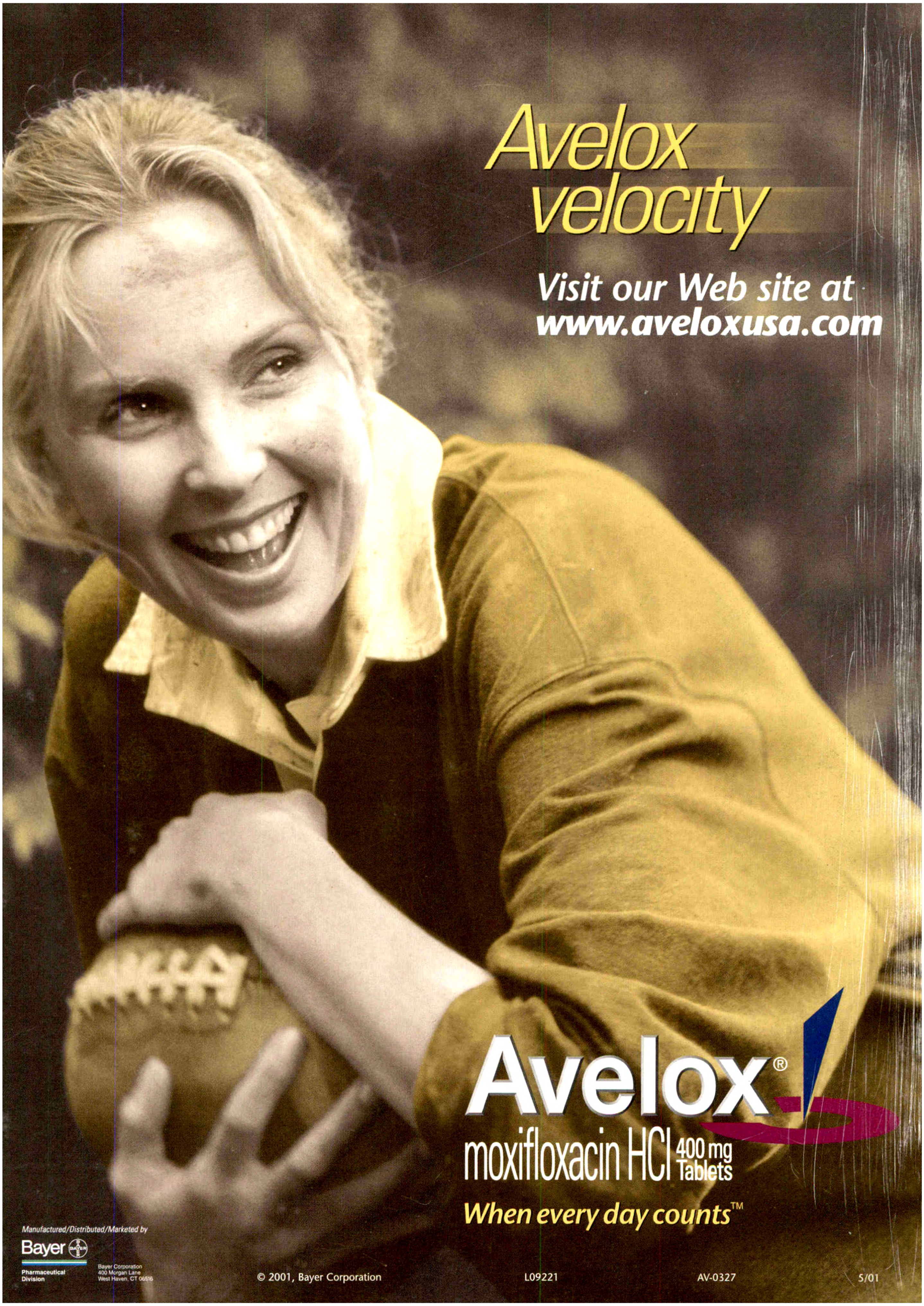
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OLUME 110 (9) — SEPTEMBER 2001

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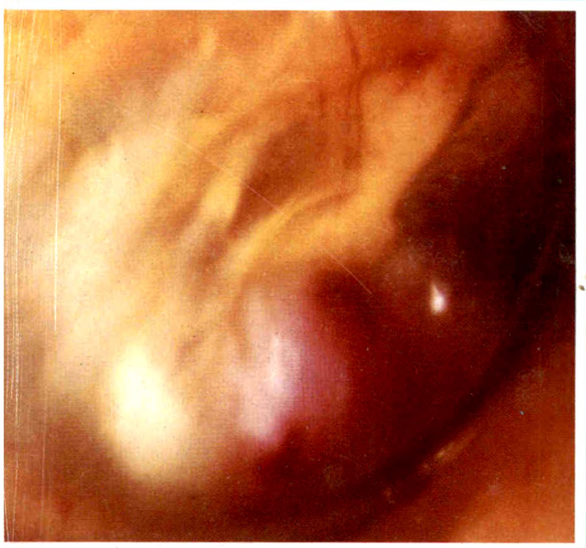

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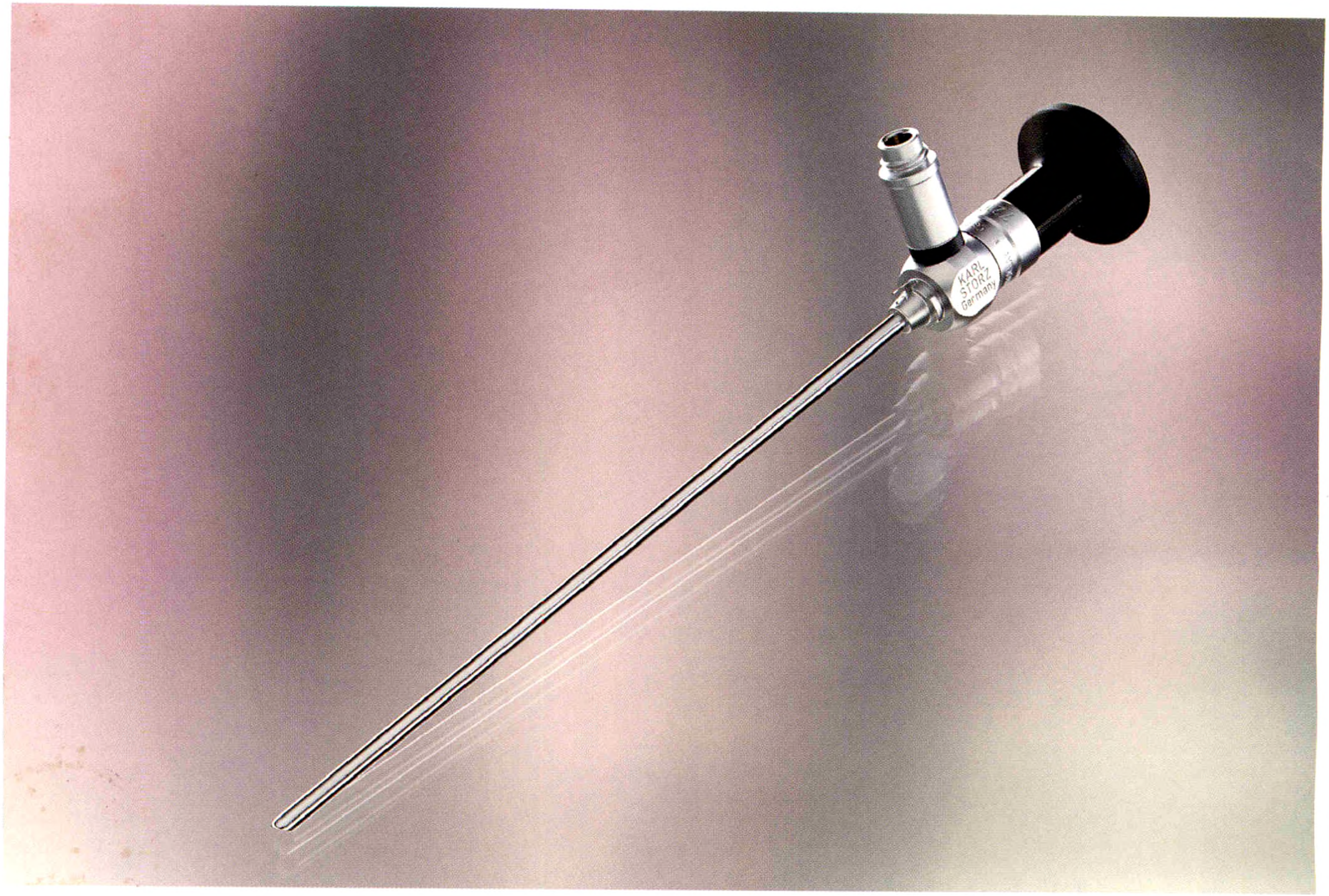

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ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY (ISSN 0003-4894) is published monthly by Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108. Periodicals postage paid at St Louis, Mo. POSTMASTER: Send address changes to *ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY*, c/o Annals Publishing Company, 4507 Laclede Avenue, St Louis, MO 63108-2103.

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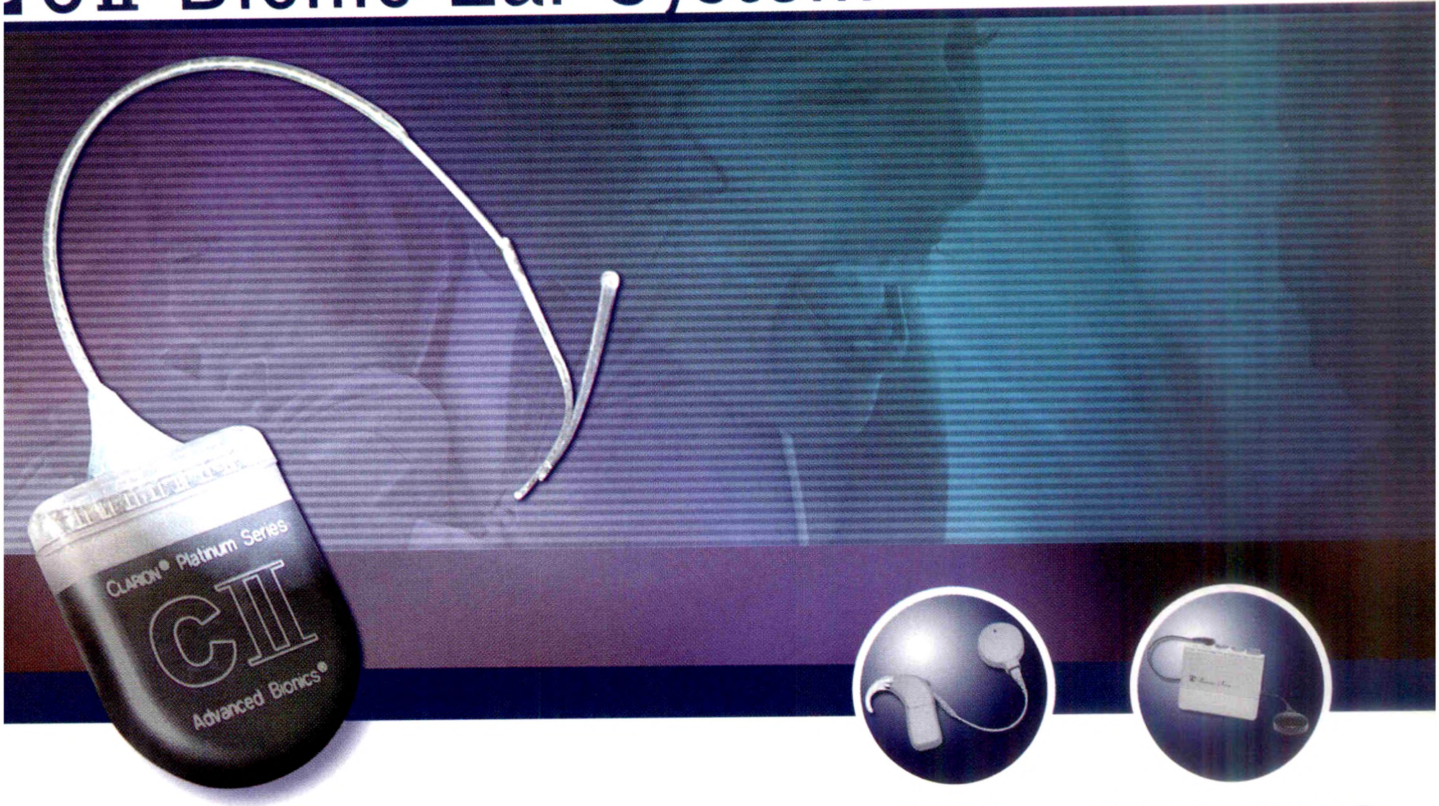

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Ann Otol Rhinol Laryngol 110:2001

MOTION-SPECIFIC LARYNGEAL REINNERVATION USING MUSCLE-NERVE-MUSCLE NEUROTIZATION

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There is no current treatment method that can reliably restore physiologic movement to a paralyzed vocal fold. The purposes of this study were to test the hypotheses that 1) muscle-nerve-muscle (M-N-M) neurotization can be induced in feline laryngeal muscles and 2) M-N-M neurotization can restore movement to a paralyzed vocal fold. Muscle-nerve-muscle neurotization can be defined as the reinnervation of a denervated muscle via axons that are induced to sprout from nerves within an innervated muscle and that then traverse a nerve graft interposed between it and the target denervated muscle. A paralyzed laryngeal muscle could be reinnervated by axons from its contralateral paired muscle, thus achieving motion-specific reinnervation. Eighteen adult cats were divided into sham, hemilaryngeal-denervated, and M-N-M–reinnervated thyroarytenoid muscle groups. Five of the 6 reinnervated animals had histologic evidence of axons in the nerve graft, 4 of the 6 had evoked electromyographic evidence of crossed reinnervation, and 1 of the 6 had a return of appropriately phased adduction. This technique has great potential and should be further investigated.

KEY WORDS — cat, laryngeal reinnervation, larynx, muscle-nerve-muscle neurotization, vocal fold paralysis.

INTRODUCTION

Situated at the crossroads of the respiratory and digestive tracts, the human larynx serves 3 important functions: 1) it makes up a part of the airway, 2) it protects the airway from soiling by food or secretions, and 3) it is the source of voice or phonation. Precise and appropriate coordination of intrinsic laryngeal movements with the phases of respiration, swallowing, and voluntary communication is essential for normal laryngeal functioning.

Unilateral vocal fold paralysis (UVFP) is a common clinical problem faced by the laryngologist. In most cases of UVFP, the phonation is grossly abnormal, and the protective laryngeal function is also sometimes impaired. The fundamental defect causing dysphonia is the lack of vocal fold adduction and decreased tension, which in turn have an adverse effect upon the glottic vibratory dynamics. Abduction of the paralyzed vocal fold is also absent, but this is rarely of clinical significance when the contralateral vocal fold has normal mobility.

Restoration of physiologic motion to a paralyzed vocal fold is not routinely possible. After denervation of skeletal muscle in general, the best reconstructive option is nerve repair to provide for direct reinnervation of the denervated muscles and resumption of contractile function. Remarkably, the intricate muscle-nerve interaction necessary for voluntary animation of striated muscles can be reconstituted via reinnervation. It has previously been established that reinnervated muscles can regain most of their mass and can, depending on the circumstances, regain the majority of their contractile capacity.[1-3] Direct nerve repair of the recurrent laryngeal nerve (RLN) yields poor reconstructive results, because both abductor and adductor fibers exist in this nerve, and anomalous axonal regeneration occurs through the repaired nerve. The resulting lack of appropriately coordinated contraction of muscle groups leads to synkinesis.[4,5]

Treatment for UVFP is typically designed to improve the voice, and reviews of the various treatment options have been published.[6,7] Injection laryngoplasty and laryngeal framework surgery are the most commonly performed surgical procedures for voice rehabilitation. Although usually beneficial,[8] these

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Supported in part by a grant from the American Laryngological Voice Research and Education (ALVRE) Foundation. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Veterans Affairs Medical Center, Ann Arbor, Michigan. This material is based upon work conducted in part in the research facilities of the Veterans Affairs Medical Center, Ann Arbor, Michigan.

Presented at the meeting of the American Laryngological Association, Palm Desert, California, May 12-13, 2001.

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only provide a static geometric solution for a disorder of dynamic laryngeal function. A variety of reinnervation procedures can also be employed, and laryngeal pacing is in the experimental stage.[9-21] Most nerve transfer operations have primarily sought to achieve bulk and tone in previously denervated laryngeal muscles, although some investigators have seen limited vocal fold movement after a nerve transfer. In some cases, transfer has been with nerves of firing patterns similar to those of laryngeal nerves, and in other cases, the pattern of neural input has been very different. As with framework surgery or injection laryngoplasty, good voice improvement can frequently be accomplished with a nerve transfer. With the potential exception of laryngeal pacing, though, none of these reinnervation options provides stimuli to the affected laryngeal muscles with the precise timing of the native innervation.

The broad long-term objective of this research is to improve the reconstruction of motor deficits resulting from unilateral denervation of laryngeal muscles. An overall assumption of this work is that motion-specific reinnervation of laryngeal muscles is preferable to reinnervation of the muscles via motor nerves directed to other movements (ie, nerve transfers). Motion-specific reinnervation is defined as neural input to a given muscle from pathways directed to identical motions, thereby allowing appropriate voluntary or automatic function.

With this long-term objective in mind, the current study was designed to test the hypotheses that 1) muscle-nerve-muscle (M-N-M) neurotization can be induced in feline thyroarytenoid (TA) muscles and 2) M-N-M neurotization can restore appropriate movement to a paralyzed vocal fold. Muscle-nerve-muscle neurotization can be defined as the reinnervation of a denervated muscle via axons that are induced to sprout from motor nerves within an innervated muscle and that then traverse a nerve graft interposed between the innervated muscle and the target denervated muscle. Reinnervation of the target muscle occurs via these axons. The paired nature of laryngeal muscles provides the ideal situation for this type of neurotization in UVFP, in that the nerve graft can be placed between muscles that have identical functions but are located on opposite sides of the larynx. The relatively small size of laryngeal muscles is also ideal, in that the potential for reestablishment of clinically significant muscle contractility is much greater than would be anticipated with more bulky muscle groups.

Muscle-nerve-muscle neurotization represents a novel but largely unsubstantiated method for the reinnervation of laryngeal muscle. The only published reports of this method have been by Millesi et al.[22-24] This work reported case studies of patients with longstanding, unilateral orbicularis oris paralyses. Sural nerve grafts were harvested, and one end was implanted in the innervated orbicularis oris muscle. The nerve grafts were then tunneled across the midline, and the other end was placed in the denervated orbicularis oris muscle. Reinnervation sufficient to restore oral competence and spontaneous, bilateral animation of the orbicularis oris was obtained in 6 patients. Stimulation of the normal orbicularis oris elicited an evoked electromyography (EMG) response in the contralateral orbicularis, which had been reinnervated by M-N-M neurotization. A nerve biopsy from 1 patient demonstrated multiple, myelinated axons within the nerve graft. These results, our preliminary unpublished work in the rat larynx, and the goal of true motion-specific reinnervation of laryngeal muscles were the motivation for the current study.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PARADIGM AND DESIGN

Eighteen adult cats were randomly assigned to 1 of 3 experimental groups: 1) sham surgery exposure only (sham surgery group), 2) hemilaryngeal denervation (denervated group), and 3) hemilaryngeal denervation plus M-N-M reinnervation of TA muscle (M-N-M TA group). Six cats were used in each group. Temporally, the animals underwent an initial surgical procedure as appropriate for their assigned group. A recovery period of 6 to 8 months was allowed, after which time data were collected during a second surgical procedure, at the end of which the cats were euthanized. For each animal, data collection consisted of a battery of functional and structural analyses designed to fully assess the studied muscles, nerves, and nerve grafts. A detailed description of the methods follows.

INITIAL SURGICAL PROCEDURES

General Care and Anesthetic Technique. The animals were housed in a facility in which National Institutes of Health guidelines for animal care were strictly followed. The Animal Care Committee of the Department of Veterans Affairs Medical Center, Ann Arbor, Michigan, approved the experimental protocol. All initial surgical procedures were performed under sterile conditions. The cats were anesthetized with an intramuscular injection of ketamine hydrochloride (20 mg/kg) and xylazine hydrochloride (0.5 mg/kg). After direct videolaryngoscopy to verify normal laryngeal anatomy and function, the animals were intubated and general inhalational anesthesia was maintained with isoflurane. A midline vertical incision was used for access in each group, and after completion of the procedure, the wound was closed

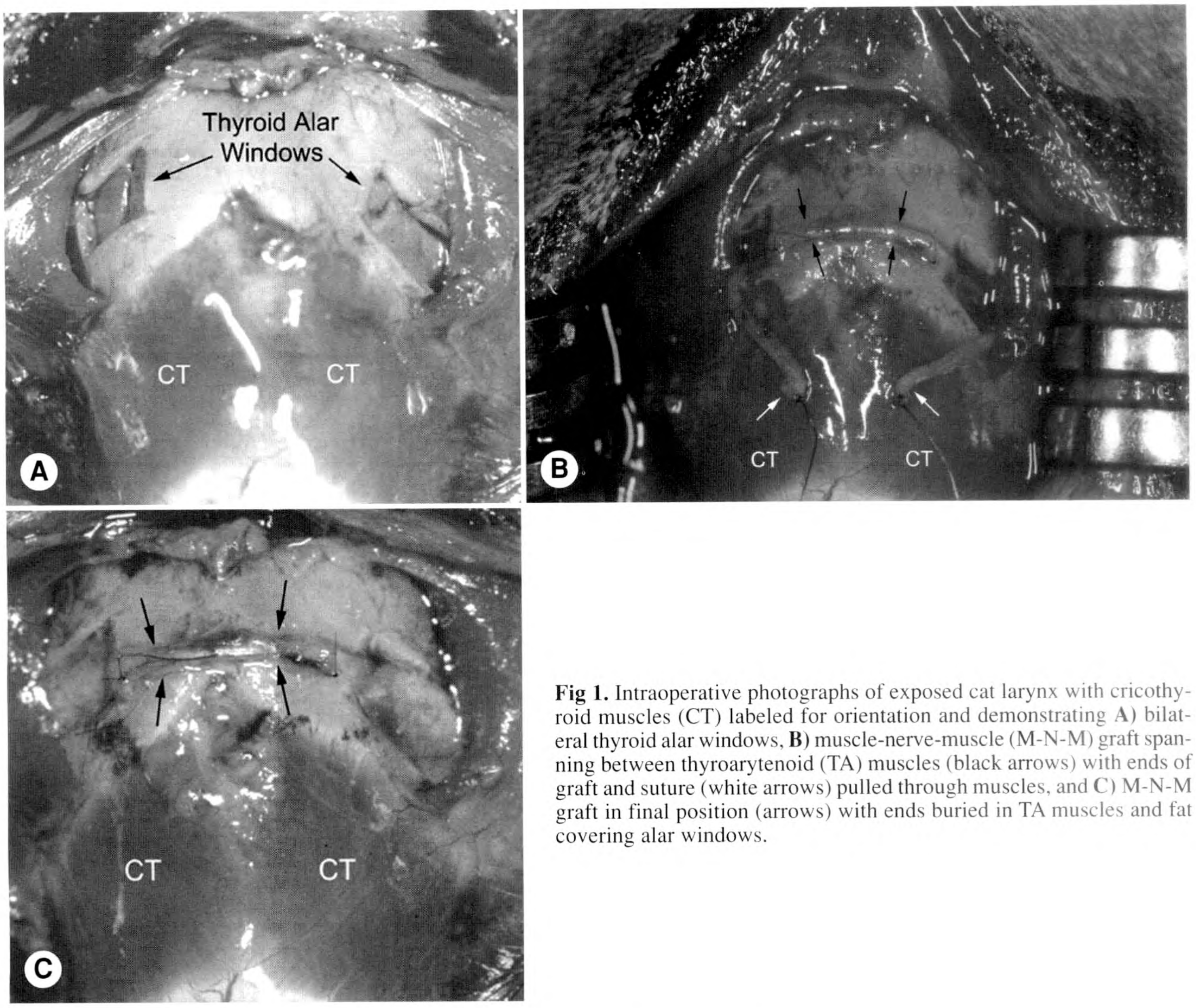

Fig 1. Intraoperative photographs of exposed cat larynx with cricothyroid muscles (CT) labeled for orientation and demonstrating **A**) bilateral thyroid alar windows, **B**) muscle-nerve-muscle (M-N-M) graft spanning between thyroarytenoid (TA) muscles (black arrows) with ends of graft and suture (white arrows) pulled through muscles, and **C**) M-N-M graft in final position (arrows) with ends buried in TA muscles and fat covering alar windows.

with 4.0 absorbable suture. After full recovery from anesthesia, the animals were housed in individual cages with food and water available ad libitum. Trained animal care workers and veterinary staff maintained close observation for signs of respiratory distress or deglutition problems.

Sham Surgery Group. The right RLN was identified, but not disturbed, in the tracheoesophageal groove. The larynx was then surgically exposed by separating the strap muscles in the midline, and the external branch of the superior laryngeal nerve (SLN) was identified near its entry into the cricothyroid (CT) muscle. Small rectangular windows of cartilage were removed from the thyroid alae bilaterally, exposing but not disturbing the investing fascia of the underlying TA muscles (Fig 1A). This step duplicated the access used in the M-N-M TA group for nerve graft placement. The alar windows were then covered with fat harvested from the suprasternal region, and the wound was closed. After wound closure, anesthesia was lightened and the cat was extubated. Direct laryngoscopy was performed during spontaneous breathing to again confirm normal vocal fold mobility.

Denervated Group. After the initial direct laryngoscopy, the right RLN and SLN were identified as in the sham surgery group. A 3- to 4-cm segment of the RLN and a 1-cm segment of the external branch of the SLN were resected, and the proximal and distal nerve stumps were cauterized by bipolar cautery. Thyroid alar windows were created as described for the sham surgery animals. After wound closure and extubation, direct laryngoscopy was performed during spontaneous breathing to confirm unilateral laryngeal paralysis.

M-N-M TA Group. After hemilaryngeal denervation and creation of thyroid alar windows as described above, a portion of the resected RLN was used for a crossed nerve graft. An 8.0 nylon suture was sewn to each end of the nerve graft, and the needle was left on the suture. One end of the graft was then buried in the left TA muscle, and the other end in the right TA muscle, by passing the needle and suture through the cartilage window anteromedially and deeply into the body of the muscle. The needle was then brought out of the muscle posterolaterally, and the graft was pulled through the muscle and then out (Fig 1B). The two ends were trimmed flush with the muscle, and gentle tension on the central body of the graft spanning between these muscles but exterior to the larynx retracted the ends of the grafts deeply into the muscle bellies. The graft was further secured with a 10.0 nylon epineural suture at the medial entry site into each muscle, and the alar windows were covered with pieces of fat (Fig 1C). After wound closure and extubation, direct laryngoscopy was performed during spontaneous breathing to confirm unilateral laryngeal paralysis.

SECOND SURGICAL PROCEDURES AND ANALYSES

We submitted a random subset of animals to brief direct laryngoscopy under intramuscular anesthesia only, to observe the vocal folds 4 to 6 months after the first operation. Six to 8 months after the initial surgical procedure, all animals underwent a second procedure and a battery of functional and structural analyses as described in the following sections.

Direct Laryngoscopy. Anesthesia was induced by intramuscular injection as previously described, while spontaneous ventilation was maintained. Spontaneous vocal fold movements were then observed via direct videolaryngoscopy with a straight-bladed pediatric laryngoscope and a 0° pediatric telescope. The animals were then intubated and general anesthesia was deepened by an inhalational agent.

Electromyography of Spontaneous TA Muscle Activity. The original surgical incision was reopened and the larynx was exposed. Spontaneous EMG recordings of the TA muscles were then made with bipolar, concentric needle electrodes. Two EMG channels were recorded simultaneously — one from each TA muscle — with an electrodiagnostic instrument (Compass Meridian, Nicolet Corporation, Madison, Wisconsin) controlled by a laptop computer (Dell Computer Corporation, Austin, Texas). Recordings were initially attempted by lightening the plane of anesthesia and placing needles into the muscles through the surgically created thyroid alar windows. It was difficult to confirm electrode placement this way, and arousal of the animal with lightening of anesthesia was also a problem. Therefore, a suprahyoid pharyngotomy approach was used, with needles placed under direct visualization into the vocal folds to record spontaneous TA muscle activity.

Electromyography of Evoked TA Muscle Activity. The left RLN was surgically exposed, and a bipolar, stimulating electrode consisting of a pair of hook-shaped wires 2 mm apart was placed around the nerve. With bilateral TA muscle electrodes in place as described above, the left RLN was stimulated at constant current with levels ranging from 0.3 to 4.3 mA. Bilateral simultaneous recordings were made from the TA muscles during stimulation of the left RLN. Amplitude and latency were calculated for the largest evoked compound muscle action potential (CMAP) recorded from each animal.

In some of the M-N-M TA animals in which a contralateral evoked TA CMAP was demonstrable, the crossed nerve graft was sectioned while stimulation and recording were continued. This was done to investigate whether, indeed, the graft was conducting the crossed CMAP activation. If this were the case, the contralateral response would disappear with division of the graft. After completion of the EMG and laryngoscopic assessments, a total laryngectomy was performed. The larynx was placed into an oxygenated physiologic solution bath. A 2-cm segment of the left RLN was harvested from each cat, and the crossed nerve graft was removed from the M-N-M TA animals. The animals were then euthanized by an intravenous injection of potassium chloride while under general anesthesia.

Histomorphometric Examination of Nerve Grafts and RLN. Nerve grafts from M-N-M TA animals and the left RLN from the sham surgery animals were harvested as described above and fixed immediately in Karnovsky's fixative. They were then postfixed in osmium tetroxide and embedded in Araldite 502. One-micron-thick, whole-nerve cross sections were cut and stained for myelin with toluidine blue. Microscopic images were digitized with an Optronics camera (Goleta, California) and a computer system (Dell). Bioquant image analysis software (R & M Biometrics, Nashville, Tennessee) was used to measure 1) the total number of myelinated axons present in each nerve or graft, 2) the area of the myelinated axons within each nerve or graft, and 3) the total area within the epineurium of each nerve or graft. The total number of myelinated axons, the average area of the individual axons, and the ratio of the sum of the individual axonal areas to the total epineural area in each specimen were used for comparisons between the M-N-M TA nerve grafts and the sham surgery RLNs.

Histomorphometric Examination of TA Muscles. The TA muscles were harvested and frozen in isopentane (2-methylbutane) and then cooled to −180°C with liquid nitrogen. Ten-micron-thick cross sections were cut from the right TA muscles on a Microm cryostat, collected on slides, and stained with hematoxylin and eosin. Microscopic images were digitized

TABLE 1. SPONTANEOUS ELECTROMYOGRAPHIC FINDINGS FOR ALL ANIMALS

Animal	*Fibrillation Right TA*	*Fibrillation Left TA*	*Polyphasic Right TA*	*Polyphasic Left TA*
Sham 1	Absent	Absent	Absent	Absent
Sham 2	Absent	Absent	Absent	Absent
Sham 3	Absent	Absent	Absent	Absent
Sham 4	Absent	Absent	Absent	Absent
Sham 5	Absent	Absent	Absent	Absent
Sham 6	Present	Absent	Absent	Absent
Denerv 1	Absent	Absent	Absent	Absent
Denerv 2	Absent	Absent	Absent	Absent
Denerv 3	Present	Absent	Absent	Absent
Denerv 4	Absent	Absent	Absent	Present, few
Denerv 5	Absent	Absent	Present	Absent
Denerv 6	Present	Absent	Absent	Absent
M-N-M TA 1	Absent	Absent	Absent	Absent
M-N-M TA 2	Present	Absent	Absent	Absent
M-N-M TA 3	Present	Absent	Present	Absent
M-N-M TA 4	Present	Absent	Present	Absent
M-N-M TA 5	Absent	Absent	Present	Absent
M-N-M TA 6	Present	Absent	Absent	Absent

TA — thyroarytenoid muscle; Sham — sham surgery group; Denerv — hemilaryngeal-denervated group; M-N-M TA — group with muscle-nerve-muscle neurotization in thyroarytenoid muscle.

TABLE 2. EVOKED ELECTROMYOGRAPHIC FINDINGS FOR ALL ANIMALS

Animal	*Amplitude CMAP L TA With Stim L RLN (mV)*	*Latency CMAP L TA With Stim L RLN (ms)*	*Amplitude CMAP R TA With Stim L RLN (mV)*	*Latency CMAP R TA With Stim L RLN (ms)*
Sham 1	18.01	1.4	0	N/A
Sham 2	1.95	2.3	0	N/A
Sham 3	10.30	2.8	0	N/A
Sham 4	6.58	3.1	0	N/A
Sham 5	2.65	3.4	0	N/A
Sham 6	8.23	3.4	0	N/A
Mean	7.95	2.73		
Denerv 1	8.35	1.6	0	N/A
Denerv 2	2.73	3.0	0	N/A
Denerv 3	3.43	2.5	0	N/A
Denerv 4	3.30	3.2	0	N/A
Denerv 5	6.74	2.1	0	N/A
Denerv 6	8.14	2.9	0	N/A
Mean	5.45	2.55		
M-N-M TA 1	3.89	1.4	0	N/A
M-N-M TA 2	6.92	2.1	0.867	3.9
M-N-M TA 3	8.56	3.9	3.600	4.9
M-N-M TA 4	7.63	2.9	1.050	4.8
M-N-M TA 5	14.0	4.1	1.490	6.8
M-N-M TA 6	4.41	2.9	0	N/A
Mean	7.57	2.88	1.75*	5.1*

CMAP — compound muscle action potential; Stim — stimulation of; RLN — recurrent laryngeal nerve; N/A — not applicable.
*M-N-M TA 2 through M-N-M TA 5.

with an Optronics camera and a computer system (Dell). The cross-sectional area from muscle fibers in four 400x fields were measured with Bioquant image analysis software (R & M Biometrics). The mean cross-sectional areas of the sham, denervated, and M-N-M TA groups were compared.

RESULTS

All animals survived the planned duration of the study with no signs of distress or failure to thrive.

Direct Laryngoscopy of Spontaneous Movements. Normal vocal fold mobility was seen bilaterally in all animals before the operations, and in the sham surgery group throughout the study. Left-sided vocal fold mobility was also normal in all cats throughout the study.

All animals in the denervated group showed right vocal fold paralysis after the initial denervation operation; none of these animals had return of appropriately phased movement that was reproducible with each respiratory cycle. Four animals had intermittent disorganized twitching movements, 1 animal had these movements plus an occasional weak adduction, and 1 had no movement.

All animals in the M-N-M TA group had a right vocal fold paralysis after the initial procedure. One reinnervated cat (M-N-M TA animal 2) had return of appropriately phased adduction of the right vocal fold during expiration. This movement was reproducible with consecutive respiratory cycles and corresponded to adduction on the left side. Intermittent twitching movements were also seen in the right vocal fold of this animal. Four animals in this group had only intermittent, disorganized twitching movements of the right vocal fold, and 1 animal had no movement.

Electromyography of Spontaneous TA Muscle Activity. The spontaneous EMG activity seen is summarized in Table 1. The necessity of general anesthesia and a pharyngotomy did not allow for reliable EMG assessments during phonation or vigorous respiration. The presence or absence of spontaneous EMG activity consisting of fibrillation or polyphasic potentials could, however, be determined. Polyphasic action potentials were present in the right TA muscle of 3 M-N-M TA animals and 1 animal from the denervated group. Fibrillation potentials from the right TA muscle were evident in 4 cats from the M-N-M TA group and 2 from the denervated group. These motor unit potentials were present in a tonic, continuous pattern.

Electromyography of Evoked TA Muscle Activity. The evoked EMG results are summarized in Table 2. In all animals, evoked CMAPs were present in the

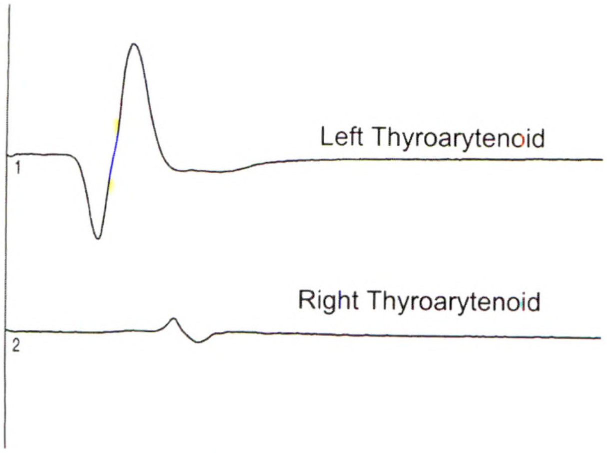

Fig 2. Simultaneous 2-channel evoked electromyographic recording of ipsilateral (left) and contralateral (right) TA compound muscle action potentials with stimulation of left recurrent laryngeal nerve for M-N-M TA animal 2.

TABLE 3. HISTOMORPHOMETRY OF NERVE GRAFTS FOR M-N-M TA GROUP

Animal	*Axon Count of Graft*	*Average Individual Axon Area (μm^2)*	*Ratio of Axon Area to Total Nerve Area*
M-N-M TA 1	0	0	0
M-N-M TA 2	75	10.35	0.0115
M-N-M TA 3	48	17.06	0.0132
M-N-M TA 4	44	5.25	0.0062
M-N-M TA 5	49	4.87	0.0052
M-N-M TA 6	6	12.46	0.0013
Mean (all animals)	37	8.33	0.0062
Mean (animals 2-6)	44.4	10.0	0.0074

ipsilateral TA muscle with stimulation of the left RLN. Contralateral TA muscle CMAPs were *not* produced in any sham surgery or denervated cats with stimulation of the left RLN. In 4 of the 6 M-N-M TA animals, both ipsilateral *and* contralateral TA muscle CMAPs were produced with stimulation of the left RLN. In each case, the contralateral CMAP had an increased latency and decreased amplitude as compared to the ipsilateral, "direct" potential. A representative evoked EMG sample for M-N-M TA animal 2 is shown in Fig 2. In 3 of these 4 animals, the nerve graft was purposely transected while we continued to stimulate and record. In each case, the contralateral CMAP immediately disappeared when the graft was transected. The ipsilateral response, however, persisted.

Histomorphometric Examination of Nerve Grafts and RLN. The nerve histomorphometry data for the nerve grafts of the M-N-M TA animals are summarized in Table 3. The left RLNs of the sham surgery animals were also studied for comparison purposes. The mean number of axons in the sham surgery RLN samples was 417, with a mean individual axon area of 61 μm^2, and a mean axonal area to total nerve area ratio of 0.54. As expected, the number and size of axons in the grafts were less than those in the native RLN. Notably, the M-N-M TA animal with restored adduction (M-N-M TA animal 2) had about 1.5 times the number of axons in the graft than the other 3 cats which demonstrated a contralateral evoked EMG but did not have restored adduction. Cross sections of the left RLN from sham surgery animal 3, from the empty graft of M-N-M TA animal 1, and from M-N-M TA animal 2 are shown in Fig 3.

Histomorphometric Examination of TA Muscles. The mean muscle fiber cross-sectional areas for the right TA muscles were 1) 888.25 μm^2 (SD, 293.53 μm^2) for sham surgery animals, 2) 807.46 μm^2 (SD, 400.46 μm^2) for denervated animals, and 3) 1,089.78 μm^2 (SD, 610.91 μm^2) for M-N-M TA animals. Representative cross sections from sham surgery animal 4, denervated animal 6, and M-N-M TA animal 5 are shown in Fig 4. There were large variations in muscle fiber areas within individual animals and within groups, but an analysis of variance found no statistically significant differences in means between groups.

DISCUSSION

As stated in the Introduction, the current study was designed to test the hypotheses that 1) M-N-M neurotization can be induced in feline TA muscles and 2) M-N-M neurotization can restore appropriate movement to a paralyzed vocal fold. Given these goals, there was a spectrum of possible degrees of success or failure anticipated when we designed the study. At one end of this spectrum would be the inability to induce any axonal sprouting at all into the crossed nerve graft, while the opposite end of the spectrum would be the successful restoration of appropriately timed contraction of the TA muscle and adduction of the reinnervated vocal fold.

The assessment methods were thus designed to accurately determine either extreme of outcome, as well as possible intermediate degrees of accomplishment. In the M-N-M TA group, histologic examination of the crossed nerve graft for myelinated axons measured the minimum level of success. Evoked EMG studies answered the crucial intermediate question of whether an electrophysiological connection was established between the two sides of the larynx, while also establishing whether this connection existed in sham surgery or denervated animals. Direct magnified videolaryngoscopy determined vocal fold movement. Spontaneous EMG data and histologic analyses of the involved muscles provided useful information, but were not really valuable in separating the

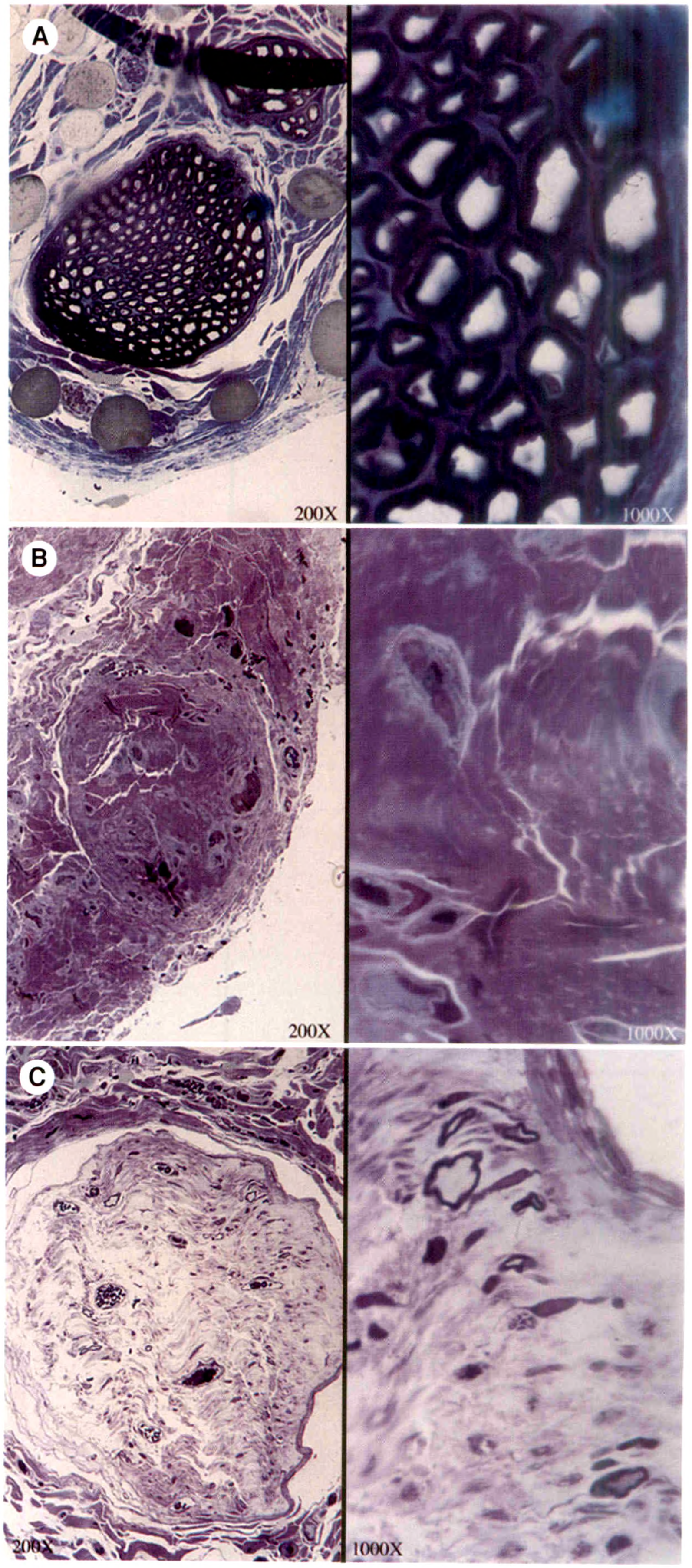

Fig 3. Histologic cross sections viewed at 200× and 1,000× from **A)** left recurrent laryngeal nerve of sham surgery animal 3, **B)** nerve graft of M-N-M TA animal 1, and **C)** nerve graft of M-N-M TA animal 2. Sham surgery animal has many large-diameter axons, and M-N-M TA animal 1 has none. Multiple myelinated axons are readily seen on high-power view of M-N-M TA animal 2.

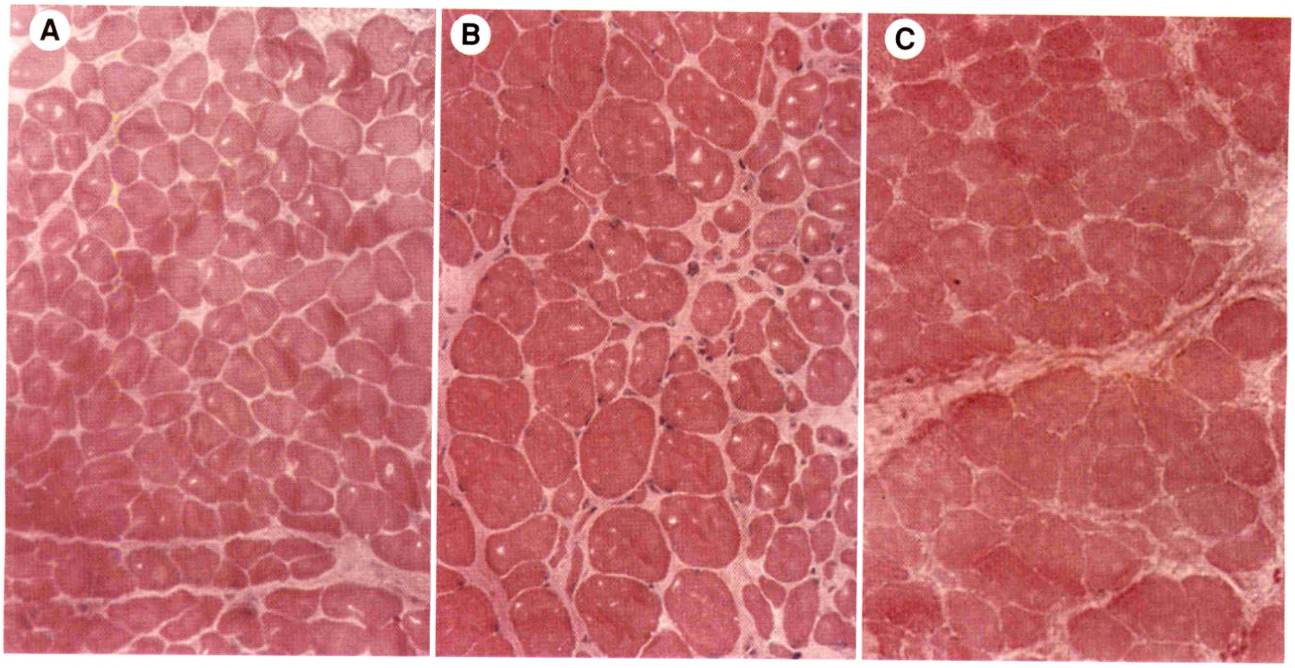

Fig 4. Representative histologic cross sections of right TA muscles from **A)** sham surgery animal 4, **B)** denervated animal 6, and **C)** M-N-M TA animal 5.

experimental groups.

All but 1 of the M-N-M TA animals achieved at least the minimum level of success, as myelinated axons were seen in the crossed nerve graft in 5 of the 6 cats (Table 3). The number of axons ranged from 6 to 75, and the mean for all 6 cats was 37. The mean for the 5 animals with axons was 44. We are unable to determine why the procedure was unsuccessful in 1 animal, or why the numbers of axons were variable. One possible factor we considered was the age of the animal; however, this information is not known.

The evoked EMG data (Table 2 and Fig 2) were extremely encouraging, and we think that these constitute the most compelling motivation for continued investigation of M-N-M reinnervation of the larynx. The fact that no measurable contralateral evoked TA CMAP existed for the sham surgery or denervated groups with stimulation of the left RLN confirms the validity of using the cat model for crossed reinnervation studies. In contrast to this finding, demonstration of both an ipsilateral *and* a contralateral evoked TA muscle response in 4 of 6 M-N-M TA cats (67%) with stimulation of the left RLN proves that our first hypothesis is true in a majority of the experimental animals. The latency and morphology of the contralateral responses as compared to the ipsilateral responses are each consistent with crossed reinnervation. Furthermore, the fact that the contralateral response immediately disappeared in all animals in which the nerve graft was sectioned during real-time EMG recording (attempted in 3 animals) is unequivocal evidence that the crossed impulses were being carried by the M-N-M graft. There also was excellent correlation between the histologic and EMG data, in that the cats with the lowest number of axons in the crossed nerve graft, 0 or 6 axons, were the ones with no contralateral evoked EMG response.

We used direct videolaryngoscopy for testing of the second hypothesis and assessment of what we believed would be the greatest potential degree of success: return of right vocal fold movement. One of the 6 M-N-M TA animals (No. 2) had this level of success. Prolonged laryngoscopy of this animal, once it was in a shallow enough depth of anesthesia for regular spontaneous respiration, showed that the right vocal fold movement was reproducible over consecutive respiratory cycles. It was synchronized with the left-side movement, with perhaps the slightest perceptible onset delay, and the magnitude of movement was less than that of the left side. The decreased magnitude would, of course, be expected even with optimum reinnervation, given that the lateral cricoarytenoid muscle, and not the TA muscle, is the strongest adductor of the vocal fold. There were twitching movements of this right vocal fold, as well, which indicated a degree of random neuromuscular activity in addition to the purposeful activity resulting in the coordinated movement. This was one of the randomly chosen animals that underwent a brief laryngoscopy about 4 months after the initial procedure, and this finding preceded the final procedure by about 2 months. That the twitching movements were more evident at 6 months than at 4 months suggests that the random reinnervation was ongoing during that interval and that the M-N-M reinnervation had occurred more rapidly.

No other animal in the M-N-M TA group or the denervated group demonstrated this same consistent right vocal fold adduction during laryngoscopy. When movement was seen in other animals from these two groups, it was primarily disorganized twitching that would be consistent with a degree of spontaneous synkinetic reinnervation. One denervated animal had twitching movements together with rare and weak adductions that were not reproducible with each respiratory cycle despite prolonged observation. We would attribute these rare movements to firing of spontaneously reinnervated muscle fibers, or possibly, interarytenoid muscle activity. Thus, a review of all the videolaryngoscopy data supports the conclusion that the second hypothesis was true in the 1 M-N-M TA animal.

The spontaneous EMG and histologic data for the right TA muscles from M-N-M TA and denervated animals indicate that a degree of reinnervation had occurred in each case. This conclusion is based on the presence of spontaneous EMG activity in 3 of the 6 denervated cats and 5 of the 6 M-N-M TA cats, together with a lack of frank post-denervation atrophy and fibrosis in any muscle. In the M-N-M TA group, it is interesting to note that polyphasic potentials, considered indicative of ongoing reinnervation, were only present in animals that also had bilateral evoked EMG activity. These potentials were also evident in 1 of the denervated cats, however. Clearly, there is a degree of spontaneous, random reinnervation of the TA muscles occurring, in addition to that which can be attributed to M-N-M neurotization. The phenomenon of subclinical reinnervation in an immobile vocal fold has, of course, been well documented.[4,5,25] In cases of neural injury in which the gross structure of the RLN is intact, the nucleus ambiguus is the source of the regenerated axons. The misdirected growth of abductor and adductor fibers leads to synkinesis rather than purposeful movement. In cases of complete nerve transection, as in this study, the source of the reinnervation is much less clear, although there is evidence in the literature to support an autonomic origin for these nerves.[26,27] We are not able to determine the source of this spontaneous reinnervation from our present data.

CONCLUSIONS

The precise timing of vocal fold movements and the subtle alterations in muscle tensions that characterize normal laryngeal function are governed by complex patterns of neuromuscular control that cannot be easily duplicated. Multiple good treatments for UVFP exist, but the ability to reliably restore true physiologic movement to a paralyzed vocal fold remains elusive. Through the use of neural input from the contralateral, normal side of the larynx, the broad long-term objective of this research is to improve the reconstruction of motor deficits resulting from unilateral denervation of laryngeal muscles.

This study was designed to test the hypotheses that 1) M-N-M neurotization can be induced in feline TA muscles and 2) M-N-M neurotization can restore appropriate movement to a paralyzed vocal fold. The first hypothesis was true in a majority of experimental animals, and the second was true in 1 animal. These results are very encouraging and warrant further investigation into this technique of laryngeal reinnervation. Future research will seek to better understand the fundamental biology of M-N-M neurotization, to enhance the efficiency of this process, and, ultimately, to apply this technique in patient care.

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Ann Otol Rhinol Laryngol 110:2001

REGRESSION OF LARYNGEAL DYSPLASIA AFTER SERIAL MICROFLAP EXCISION

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There is no consensus on the ideal management of premalignant lesions of the larynx. Published reports describe the use of mucosal stripping, microsurgical techniques, CO_2 laser excision and ablation, and even conservation laryngeal surgery and radiotherapy. We performed a retrospective evaluation of 43 men and 11 women who underwent serial excision of dysplastic lesions with the microflap technique between 1990 and 2001. The average age of the patients was 57.2 years; 64% had a history of cigarette smoking, and 46% a history of alcohol consumption. The lesions were located on the middle part of the left vocal fold in 65% and on the mid-portion of the right vocal fold in 66%. The patients were followed for an average of 4.4 years. Of the 20 patients with severe dysplasia or carcinoma in situ treated with the microflap technique, only 1 progressed to invasive disease. Overall, there was a reduction in the severity of dysplasia after each procedure ($p = .0008$). The microflap technique reliably reduced the severity of dysplastic lesions of the vocal fold and was effective in local disease control.

KEY WORDS — carcinoma, laryngeal dysplasia, larynx, microflap excision.

INTRODUCTION

The development of thickened laryngeal mucosa associated with superficial keratosis is commonly referred to as leukoplakia. The potential for malignant degeneration has been linked to altered vertical cellular organization and structural and nuclear changes within the cell. According to this potential, 3 levels of histologic changes can be classified: hyperplasia, an increased density of cells without any change in maturation; metaplasia, in which the epithelium is transformed; and dysplasia, which is a maturational abnormality with cellular atypia and disorder.[1]

It has been postulated that the majority of cases of dysplasia occur in response to irritants, including tobacco smoke, gastroesophageal reflux, mechanical trauma, some inhaled chemicals, and alcohol, which has been postulated to cause chronic vascular engorgement and inflammatory response.[2,3] Current methods for grading squamous dysplasia of the vocal folds remain somewhat subjective, and although more than 20 separate classification systems have been published to date, no single system is widely accepted.[4] This lack of consensus has led to some difficulty with clinical decision-making and highlights the importance of communication between surgeon and pathologist. The histopathologic parameters considered to be most helpful in determining dysplasia grade are abnormal mitotic figures, mitotic activity, maturation level, nuclear pleomorphism, and stromal inflammation.[5]

Higher levels of dysplasia are believed to exhibit increasing potential for malignant degeneration.[6] Management of patients who exhibit significant degrees of laryngeal dysplasia remains controversial. Treatment options include endoscopic stripping of the affected mucosa with or without CO_2 laser ablation, partial laryngectomy, and external-beam radiotherapy.

The medial microflap procedure was described by Courey et al[7] as a minimally invasive means of excising laryngeal disease while preserving vocal function. In this paper we describe the application of the microflap technique in the treatment of laryngeal dysplasia and review our treatment results along with those of more traditional techniques.

MATERIALS AND METHODS

We performed a retrospective review of 54 consecutive patients treated for laryngeal dysplasia or carcinoma in situ (CIS) between 1990 and the present. Patients with a history of laryngeal carcinoma or radiotherapy to the larynx were excluded. Information regarding patient age, sex, exposure to alcohol and

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Presented at the meeting of the American Laryngological Association, Palm Desert, California, May 12-13, 2001.

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tobacco, history of gastroesophageal reflux, and vocal use was obtained. Patients were asked to discontinue tobacco and alcohol consumption and were treated for reflux if such treatment was indicated. All patients underwent preoperative videostroboscopy both for photodocumentation and for analysis of vocal fold mobility and integrity of the mucosal wave. The type, timing, and glottic location of each surgical intervention were recorded, as well as the pathological diagnosis of each specimen removed. Surgical procedures included simple biopsy, traditional vocal fold stripping, microflap excision, and CO_2 laser ablation of the affected area. All surgical procedures were performed with the patient under general endotracheal anesthesia with paralysis; a Teflon-coated, wrapped, laser-safe endotracheal tube and a standard suspension microlaryngoscope were used.

The microflap procedure is performed by making an incision adjacent to the lesion. The mucosa including the lesion is then elevated off the deep layer of the lamina propria and excised, with care taken not to violate the vocalis muscle. If the lesion is adherent or if the underlying mucosa appears otherwise involved, a deep biopsy specimen is taken and the area is further excised or ablated with the laser at a power setting of 2 to 4 W with the beam defocused.

Patients were followed monthly after diagnosis and returned to surgery if there was any evidence of recurrent disease. Patients with a diagnosis of severe dysplasia or CIS were returned to surgery for repeat biopsy or microflap excision as soon as any gross postoperative inflammation had resolved, typically in 4 to 6 weeks. Any changes noted or suspected in follow-up were again documented and tracked by videostroboscopic examination. Patients were returned to surgery at regular intervals until any severe dysplastic lesions had cleared.

The specimens used for statistical analysis were processed by permanent whole-mount serial section and were studied by in-house pathologists. For the anatomic location of each specimen, the larynx was divided into 7 subsites, including the anterior commissure and the right and left anterior, middle, and posterior parts of the vocal folds.

RESULTS

The group consisted of 43 men and 11 women. The average age at presentation was 57.2 years. The average age of the group at last follow-up was 59 years. With respect to known risk factors for dysplastic lesions of the larynx, 64% of the group used tobacco regularly, 46% percent of the group reported regular use of alcohol, 48% had a diagnosis of gastroesophageal reflux, and 7% had evidence of chronic vocal abuse. The mean follow-up period for the group was 4.4 years. At presentation, the anterior part of the left vocal fold was involved in 30.9% of the patients, the anterior part of the right fold in 29%, the middle part of the left fold in 54.4%, the middle part of the right fold in 63%, the posterior part of the left fold in 16.1%, and the posterior part of the right fold in 6.3%. The anterior commissure was involved in 19.7% of the patients. The prevalence of the involvement of the middle part of the fold as opposed to other glottic sites was statistically significant ($p = .04$).

Four patients (7.7%) who presented with apparent leukoplakia had index pathological specimens without dysplasia, 14 patients (25.9%) had specimens with mild dysplasia, 14 (25.9%) had specimens with moderate dysplasia, 8 (14.8%) had specimens with severe dysplasia, and 14 (29.8%) had specimens with CIS.

A total of 162 lesions were removed, for an average of 3.0 per patient. The microflap technique was used for 137 lesions, and vocal fold stripping for 25. The laser was used along with vocal fold stripping in 3 cases. The microflap technique resulted in a decrease in the grade of dysplasia by 69% of a single grade (eg, moderate to mild dysplasia; $p = .0008$). Vocal fold stripping resulted in an apparent increase in grade by 68% on subsequent procedures ($p = .19$). In 20 patients with severe dysplasia or CIS, 98 microflap excisions were performed on a total of 24 lesions. The laser was used in 7 cases. One of the 20 patients with severe dysplasia or CIS experienced progression to invasive disease ($p = .002$). Thirty-six patients had no evidence of leukoplakia at last follow-up.

DISCUSSION

It is well established in the literature that advancing dysplasia is associated with increasing malignant potential.[2,8,9] The appropriate management of these lesions remains controversial. Many series have described the use of radiotherapy and partial laryngectomy in the management of severe intraepithelial neoplasia without invasion.[10,11] Small et al[11] reviewed the literature for treatment of patients with severe dysplasia or CIS. Out of 415 patients who underwent initial radiotherapy in 14 studies, 20% had a recurrence; 73% to 100% of the recurrences involved invasive disease. Forty percent of patients who underwent endoscopic CO_2 laser surgery in 6 studies and vocal fold stripping in 8 studies had recurrences, but with a significantly lower incidence of invasive disease. The authors concluded that laser ablation represented the best chance for cure, on the basis of a recurrence rate of 8%.

In the present study, the addition of laser treatment did not offer a statistically significant improvement over microsurgical treatment alone, although the number of uses was small. Using laser excision of a mucosal flap and deep ablation as their primary treatment method, McGuirt and Browne[12] achieved 100% ultimate local control of CIS and microinvasive disease over a 4-year follow-up period. Twenty-four percent of their patients had 1 or more local recurrences that required repeat excision. Wolfensberger and Dort[13] pointed out the limitations of laser dissection at the anterior commissure, however, noting a 50% recurrence rate.

Regardless of treatment, the literature suggests a much higher rate of progression to invasive squamous cell carcinoma in patients with severe dysplasia and CIS. In the present study, only 1 patient with a presenting pathological diagnosis of severe dysplasia or CIS treated with the microflap technique progressed to invasive disease. The microflap technique reliably reduced the degree of dysplasia on subsequent procedures ($p = .0008$) and was an effective means of treating these high-risk patients.

Several factors may explain the apparent success of the microflap technique in the treatment of dysplasia. The technique allows for precise microscopic control of the area of resection, and the delineation between healthy and abnormal tissue may be more clearly identified. This is important not only for precise control of margins, but also because the surrounding tissue is left untouched. On subsequent office visits, the area in question is more easily identified, and any changes may be noted by indirect laryngoscopy and confirmed by videostroboscopy. This improved specificity is also helpful at the time of repeat direct laryngoscopy. In addition to precise control, vocal function is maximally preserved. Conversely, vocal fold stripping results in a less controlled area of resection, with more generalized scarring that can obscure the original site of the lesion. It is possible that diffuse fibroblastic activity may envelop dysplastic cells, resulting in submucosal spread of disease and difficulty in following the original lesion. Last, minimal mucosal resection maximizes vocal function, which can be compromised by excessive surrounding tissue loss with resultant fibrosis and loss of the mucosal wave. Laser treatment may also induce scarring similar to that of stripping, and the voice outcome may be compromised more than with simple excision, especially after repeat procedures.

Several arguments can be made against early irradiation for laryngeal squamous dysplasia. Although individual lesions may be curable with radiotherapy, the remainder of the treated field cannot be sterilized against the development of future lesions.[14] In addition, one treatment arm has been eliminated from future use, and any recurrence will thus obligate the use of radical surgical salvage. Certainly, many patients may suffer local side effects from adequately dosed irradiation, including xerostomia, pain, dysphonia, and osteoradionecrosis. The cost of a 5- to 6-week course of radiotherapy exceeds that of 3 separate endoscopic procedures. Finally, studies have shown that radiotherapy may actually promote the long-term development of invasive disease from dysplastic epithelium.[15,16]

The need for close follow-up and repeat endoscopic procedures has been recognized by other authors. In their review of 20 patients with CIS and microinvasive disease, Rothfield et al[17] concluded that ultimate disease control was more contingent on follow-up than on the type of treatment. The 2 treatment failures cited occurred at 64 and 65 months, after the patients had been lost to follow-up for 4 years. Despite good ultimate disease control, patients required as many as 5 procedures each, with an overall average of 2.25.

Many authors cite difficulty in comparing treatment results between institutions because of potential differences in pathological diagnosis of grades. However, out of 148 specimens reported by Blackwell et al,[5] pathologists evaluating the same specimen differed by more than 1 histologic grade only 8 times. In addition, slides read by teams of pathologists are more comparable than those reviewed by a single pathologist, because intragroup discrepancies are likely to be similar from group to group. Similarly, having all of the specimens evaluated by a single pathologist maximizes intragroup consistency, but these results may not be comparable across other groups because of individual bias. In addition, many authors believe that the malignant potential of severe dysplasia is equal to that of CIS, if not greater, in which case stepwise discrepancies between individual grades may not be critical to discern.[2,5,18]

The finding of a male sex predominance (85%) in the present study confirms previous studies.[5] In addition, a minority of patients had posterior glottic involvement. In their landmark article, Ward and Hanson[3] found that 14 of 19 patients with no significant risk factors except a history of gastroesophageal reflux disease had posterior glottic lesions. Neither their series nor the present series established the diagnosis of reflux with definitive diagnostic tests; however, the incidences of posterior glottic involvement are similar. There was no statistical correlation between alcohol use or voice abuse and dysplasia, but the number of patients with voice abuse in the present series was relatively small.

CONCLUSION

We have had excellent results with the microflap technique in the treatment of laryngeal dysplasia. In our experience, this technique provides an optimal combination of surgical precision and local control, improved ability for close follow-up, and optimal vocal function through maximal mucosal preservation. Although the early results have been promising, continued follow-up is needed to realize the long-term results. When using any type of local resection, a clinician must maintain diligent follow-up and be willing to perform repeat endoscopy as necessary. Until proven otherwise, risk factor modification and medical therapy should also be considered important parts of the overall management of this disease.

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Ann Otol Rhinol Laryngol 110:2001

LACK OF EVOKED LARYNGEAL ELECTROMYOGRAPHY RESPONSE IN PATIENTS WITH A CLINICAL DIAGNOSIS OF VOCAL CORD PARALYSIS

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There has been recent debate about whether patients with vocal cord immobility have a neurologic paralysis or whether synkinesis, the misdirection of axons to competing laryngeal muscles, is responsible for the lack of voluntary vocal cord motion. This issue was studied in 15 patients with vocal cord paralysis who underwent laryngeal reinnervation. Evoked electromyography was performed with a surface electrode endotracheal tube. The recurrent laryngeal nerve (RLN) was identified and stimulated with constant current. Of the 15 patients, only 1 produced a compound muscle action potential upon nerve stimulation. The remaining 14 patients had no evoked response during RLN stimulation. A control group of 8 patients with normal vocal cord mobility was studied, and each had a normal evoked electromyography response after RLN stimulation. These results support the assertion that patients who require treatment for vocal cord paralysis do not have synkinesis produced by RLN reinnervation.

KEY WORDS — electromyography, larynx, paralysis, synkinesis, vocal cord.

INTRODUCTION

Vocal cord paralysis may be secondary to surgical trauma, invasion of the recurrent laryngeal nerve (RLN) by cancer, or penetrating trauma to the neck or chest. In most cases, it is idiopathic. The adopted position of the vocal cord has been a long-standing matter of debate and is thought to reflect the underlying cause of the paralysis.[1] Wagner[2] and Grossmann[3] proposed that isolated RLN paralysis caused the vocal cord to adopt a paramedian position. Combined superior laryngeal nerve (SLN) and RLN paralysis produced a more lateralized position, presumably secondary to the loss of adductory function by the cricothyroid muscle.[2,3] In contrast, Rosenbach[4] and Semon[5] proposed that vocal cord paralysis represented a progressive dysfunction of abductory nerve fibers first, followed later by adductory nerve fibers.

It has been postulated more recently that many patients presenting with a diagnosis of unilateral vocal cord paralysis may actually be demonstrating synkinesis, rather than a truly paralyzed vocal cord.[6] Synkinesis is the misdirected and inappropriate reinnervation of the laryngeal muscles by nerve fibers of the RLN, which results in neuromuscular discoordination and simultaneous contraction of abductor and adductor muscles.[6] An examining otolaryngologist may therefore mistake a cord fixed in position by the simultaneous contraction of its abductors and adductors for a paralyzed vocal cord.

Fifteen patients with a diagnosis of vocal cord paralysis were referred to UCLA Medical Center for evaluation and treatment. Patients with unilateral vocal cord paralysis underwent arytenoid adduction and simultaneous anastomosis of the RLN to a branch of the ansa cervicalis. Patients with bilateral vocal cord paralysis underwent unilateral transection of the thyroarytenoid muscle nerve branch and reinnervation of the posterior cricoarytenoid muscle. Before reinnervation, each patient underwent evoked electromyography (EMG). This article summarizes the results. Only 1 patient with a diagnosis of vocal cord paralysis demonstrated evoked EMG activity with stimulation of the RLN. Evoked EMG in the remaining patients showed no activity in the musculature on the paralyzed side.

PATIENTS AND METHODS

Fifteen patients presented to UCLA Medical Center with a diagnosis of vocal cord paralysis: 13 with unilateral vocal cord paralysis and 2 with bilateral vocal cord paralysis (Table 1). The duration of paralysis ranged from 2 months to 17 years (mean, 3 years 4 months; median, 1 year 2 months). There were 7 men and 8 women. Three patients had received collagen injection previously. One patient had undergone previous type I thyroplasty. All 13 patients with unilateral vocal cord paralysis complained of a hoarse, breathy voice and fatigue with prolonged voice use. Both patients with bilateral vocal cord paralysis com-

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Presented at the meeting of the American Laryngological Association, Orlando, Florida, May 13-14, 2000.

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TABLE 1. SUMMARY OF PATIENTS PRESENTING WITH VOCAL CORD PARALYSIS

Patient	*Age (y)*	*Sex*	*Side*	*Duration*	*Cause*	*Prior Therapy*
1	42	M	Left	1 y 1 mo	Aortic laceration	Collagen injection ×1
2	47	M	Left	8 y 7 mo	Idiopathic	Collagen injection ×2
3	29	F	Both	2 y 7 mo	Total thyroidectomy	
4	70	M	Left	8 mo	Total thyroidectomy	
5	44	F	Left	2 y	Idiopathic	
6	35	M	Right	2 y	Idiopathic	
7	25	F	Left	8 y	Idiopathic	
8	36	F	Left	1 y 9 mo	Craniotomy	
9	49	F	Left	1 y	Cervical fusion	
10	52	F	Right	17 y	Thoracotomy	Thyroplasty
11	64	M	Left	8 mo	Carotid endarterectomy	
12	44	F	Both	10 y	Total thyroidectomy	
13	60	M	Right	2 y	Sarcoidosis	Collagen injection ×3
14	55	F	Left	9 mo	Left thyroidectomy	
15	51	M	Left	2 mo	Dissecting aortic aneurysm	

plained of hoarseness and dyspnea with exertion, and were found to have mild inspiratory stridor. Ten of the 15 patients underwent a preoperative phonatory function evaluation that included evaluation of vocal cord waveform, pitch range, subglottic pressure, transglottic airflow, acoustic analysis, percent jitter, mean shimmer, glottic resistance, and harmonics-to-noise ratio.

All patients were intubated transorally with a custom-made endotracheal tube outfitted with surface electrodes capable of recording evoked laryngeal EMG responses. This technique has been reported previously.[7] After the induction of general anesthesia, the larynx was exposed through a transcervical incision with subsequent identification of the SLN and RLN. Separate stimulations of the SLN and RLN on the paralyzed side were performed with 0.1-ms square wave pulses with an intensity of 0.5 to 3 mA.

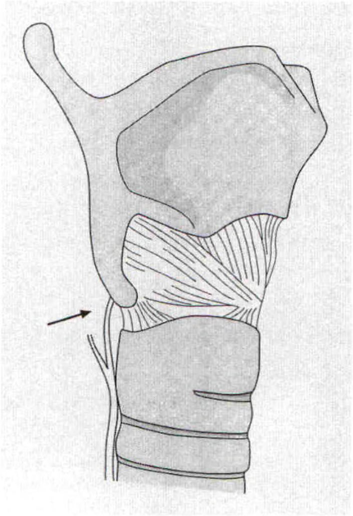

Fig 1. Relationship of recurrent laryngeal nerve to cricothyroid articulation. Nerve enters larynx 1 cm posterior and 1 cm inferior to joint (arrow).

All patients with unilateral vocal cord paralysis underwent arytenoid adduction and reinnervation of the paralyzed vocal cord with anastomosis of the RLN to a branch of the ipsilateral ansa cervicalis. This technique has been described previously.[8] Patient 3, with bilateral vocal cord paralysis, underwent transection of the thyroarytenoid muscle branch through a window made in the ipsilateral lamina of the thyroid cartilage with subsequent anastomosis of the RLN to the ipsilateral ansa cervicalis. This was done to selectively reinnervate the posterior cricoarytenoid muscle and improve her respiratory symptoms. Patient 12, also with bilateral vocal cord paralysis, also underwent transection of the thyroarytenoid muscle branch and reinnervation of the posterior cricoarytenoid muscle with an ipsilateral cricothyroid muscle pedicle. In all patients, the anatomic identity of the RLN was confirmed by following the RLN to its entrance intralaryngeally at the cricothyroid joint. In all patients, the nerve was identified at a point 1 cm posterior and 1 cm inferior to the cricothyroid articulation (Fig 1).

In 4 patients, 28-gauge bipolar concentric needle electrodes (Neurosupplies, Waterford, Connecticut) were placed into the ipsilateral thyroarytenoid muscle through the thyroid cartilage window and the ipsilateral RLN was stimulated. This step was done to confirm the identity of the thyroarytenoid muscle and also to determine whether stimulation of the RLN would activate motoneuron units within the muscle.

All patients underwent identification and stimulation of the SLN on the operative side, and the subsequent EMG waveforms were recorded. This step was done to confirm that the endotracheal tube was in place and that the surface recording electrodes were functional. In addition, a control group of 8 patients who underwent hemithyroidectomy or total thyroidectomy were also studied. All patients had normal

TABLE 2. ABNORMALITIES FOUND ON PHONATORY FUNCTION EVALUATION

Patient	*Mucosal Waveform*	*Pitch Range*	*Subglottic Pressure (cm H2O)*	*Transglottic Airflow (LPS)*	*Acoustic Analysis*	*Mean Jitter (%)*	*Mean Shimmer (dB)*	*Laryngeal Resistance (cm H2O/ LPS)*	*Harmonics-to-Noise Ratio (dB)*
1	↓	↓	↑	↑	Too aperiodic for interpretation				
2	↓	↓	WNL	↑		↑	WNL	↓	↑
3	↓	↓	↑	↑	Too aperiodic for interpretation			↓	
7			↓	WNL				↓	
8			WNL	↑	Too aperiodic for interpretation			↓	
9	Absent		WNL	WNL				WNL	
10			WNL	WNL		↑	↑	WNL	↓
11			↑	↑				WNL	
13				↑		↑	WNL	↓	WNL
15	↓	↓	↓	↑		↑	WNL	↓	WNL

Blank space indicates no data.

LPS — liters per second; ↓ — decreased; ↑ — increased; WNL — within normal limits (ie, subglottic pressure 5.5 to 11.3 cm H_2O, transglottic airflow 0.128 to 0.254 LPS, mean jitter 0.324% to 0.641%, mean shimmer 0.189 to 0.495 dB, laryngeal resistance 29.6 to 54.4 cm H_2O/LPS, harmonics-to-noise ratio 22.14 to 30.98 dB).

vocal cord motion before and after the operation. In all patients, 1 or both RLNs were identified and stimulated, and the resulting evoked EMG waveforms were compared to those of the patients with unilateral vocal cord paralysis.

RESULTS

All 10 patients who underwent preoperative phonatory function evaluation showed multiple abnormalities (Table 2). Most commonly, unilateral vocal cord paralysis produced decreased glottic resistance, which resulted in a compensatory increase in subglottic pressure and transglottic airflow.

All 8 patients who underwent hemithyroidectomy or total thyroidectomy had normal intraoperative evoked EMG waveforms with RLN stimulation (Fig 2A). In contrast, of the 15 patients with vocal cord paralysis, only 1 patient (No. 6) showed a normal evoked EMG waveform with RLN stimulation. This patient subsequently underwent ipsilateral arytenoid adduction only.

The remaining 12 patients with unilateral vocal cord paralysis showed no evoked EMG waveform with RLN stimulation (Fig 2B). Neither patient with a diagnosis of bilateral vocal cord paralysis showed evoked EMG activity in the vocal cord on the operative side. In all 15 patients, stimulation of the SLN on the operative side produced ipsilateral contraction of the cricothyroid muscle that could be seen visually and that demonstrated a characteristic EMG

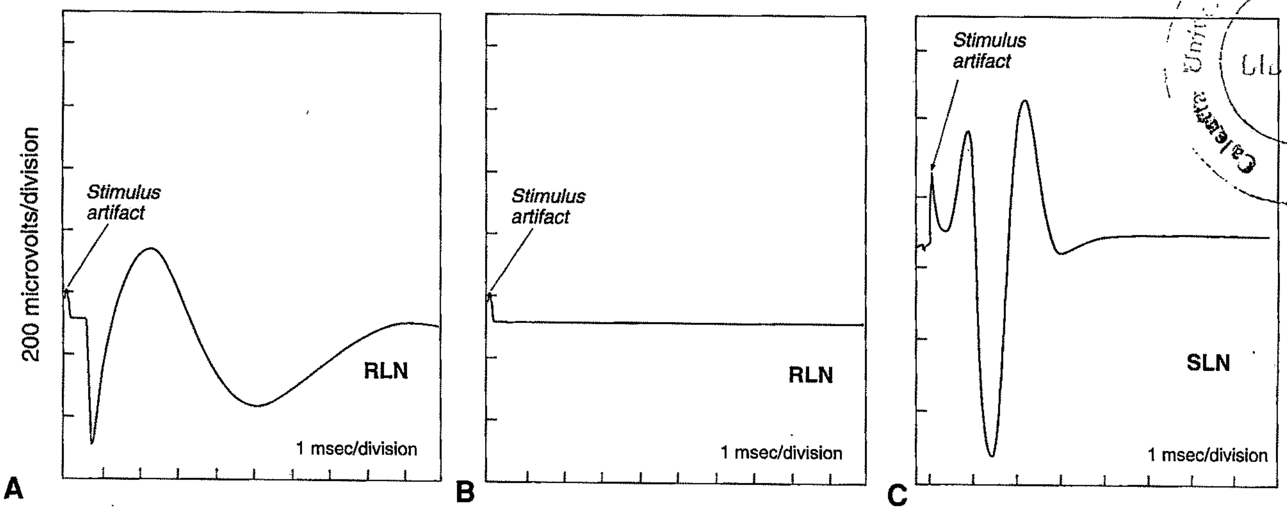

Fig 2. Electromyography. **A)** Normal evoked response from normal vocal cord after stimulation of ipsilateral recurrent laryngeal nerve (RLN). **B)** Lack of evoked response from paralyzed vocal cord after stimulation of ipsilateral RLN. **C)** Normal evoked response after stimulation of ipsilateral superior laryngeal nerve (SLN).

waveform, as well (Fig 2C). This waveform has been described previously.[7] This finding served as confirmation that the endotracheal tube was in place and functional.

Of the 4 patients in whom EMG activity in the thyroarytenoid muscle was measured with intramuscular bipolar concentric needle electrodes, no patient showed evoked EMG activity with stimulation of the ipsilateral RLN. Two patients showed electrical silence after insertion of the needle electrodes, and 2 patients showed fibrillation potentials.

DISCUSSION

Unilateral vocal cord paralysis is uncommon. The most common causes are trauma, thyroidectomy, and nonlaryngeal malignancies, and these constitute the bulk of referrals to otolaryngologists. Approximately one third of patients with idiopathic vocal cord paralysis improve, one third remain the same, and one third require intervention.

In evaluation of an immobile vocal cord, a distinction between true paralysis and immobility resulting from other factors must be made. Paralysis implies a disruption of neuromuscular innervation, whereas immobility may be caused by cricoarytenoid joint fixation, synkinesis, or myopathy.[9] Laryngeal EMG may be useful in distinguishing between these various causes and in assessing the prognosis of vocal cord paralysis.[10-12] It is thought that the phenomenon of synkinesis complicates the functional reinnervation of the paralyzed hemilarynx and that most patients may be demonstrating synkinesis, rather than true paralysis.[6]

Interestingly, despite widely varying causes of vocal cord immobility, none of these 14 patients showed evoked EMG activity with RLN stimulation or evidence of synkinesis. This finding implies either that motoneurons have not regenerated along the RLN or that they have not made functional connections to the motor end plates. In contrast to other series[10-12] that have investigated EMG patterns in the paralyzed vocal cord, the group of patients in this series underwent stimulation of the RLN under direct visualization. Stimulation of the RLN failed to produce movement or EMG activity in the ipsilateral vocal cord. Synkinesis caused by aberrant reinnervation from the RLN did not occur in these 14 patients and therefore could not account for the observed vocal cord paralysis.

The 4 patients who underwent EMG recording from the thyroarytenoid muscle with bipolar concentric needle electrodes also showed no evidence of motoneuron regeneration from the RLN. This finding also served as confirmation that the surface recording electrodes from the endotracheal tube were functioning. Because the needle electrode recordings were simply confirming our observations made with the surface recording electrodes, no further needle electrode recordings were made in the remaining patients.

The 1 patient (No. 6) who was found to have an EMG waveform with RLN stimulation had had paralysis for 3 years. Of interest is that in reviewing the videostroboscopy images obtained before operation, we saw that the right cord was actually paretic rather than paralyzed. The innervation of the cord must have been sufficient to provide a normal EMG waveform but a minimum of physiological motion.

Of all patients in this series who underwent surgery and who subsequently developed a vocal cord paralysis, only 2 (patients 1 and 15) were known to have transections of the RLN. The RLN in the remaining surgical patients was presumed to have been stretched or compressed but left intact. Thus, these patients who sustained trauma to the RLN had the same functional outcome as those patients whose vocal cord paralysis was idiopathic or atraumatic. This finding implies that there may be a common underlying pathophysiologic mechanism at work regardless of the cause of vocal cord paralysis. For example, there may be a physical obstruction somewhere along the length of the RLN or at the junction of its takeoff from the vagus nerve, perhaps from neuroma formation or more extensive scarring, that prevents motor axons from regenerating all the way back to the denervated hemilarynx.

In the evaluation of vocal cord paralysis, EMG may be a useful tool in the differentiation of synkinesis from other disorders.[10-12] For example, with synkinesis, intramuscular EMG recording from laryngeal adductor muscles may demonstrate activity during inspiration and expiration, suggesting aberrant innervation by abductory nerve fibers.[6] Patients who exhibit vocal cord paralysis after traumatic intubation may demonstrate normal EMG activity suggesting arytenoid dislocation.[13] Characteristic evoked EMG patterns may be seen with denervation injury and subsequent reinnervation, depending on the time from the onset of injury.[14] Fibrillation potentials usually denote denervation, with polyphasic potentials indicating reinnervation.

Electromyographic activity may be measured with needle or surface electrodes. Needle electrodes permit measurement of spontaneous and evoked potentials, important in the evaluation of denervation and reinnervation, whereas surface electrodes measure evoked potentials, actually, the summation potential produced by the coordinated contraction of fibers in the muscle under investigation.[15]

Prior investigations in dogs have demonstrated a motor branch from the external division of the SLN to the thyroarytenoid muscle.[16] Wu et al[17] have also confirmed the presence of this branch in 44% of human larynges. The existence of such a branch in a patient may represent an alternative route of motor reinnervation to the paralyzed thyroarytenoid muscle and may mislead the clinician in interpreting EMG data. For example, the detection of polyphasic potentials in a paralyzed thyroarytenoid muscle, although indicative of reinnervation,[14] may actually represent reinnervation from the SLN, rather than the RLN. In addition, adjacent laryngeal muscles may sprout motor branches to their denervated neighbors. This phenomenon of intralaryngeal muscular neurotization has been observed for some time.[18-20] Therefore, polyphasic reinnervation potentials may not be indicative of a pending return of physiological vocal cord function, and represent a potential pitfall in the interpretation of EMG data.

Future investigations should include recordings of evoked and spontaneous EMG activity in the thyroarytenoid muscle with bipolar concentric needle electrodes. There may be scattered motoneuron units within the thyroarytenoid muscle that receive axons either from the regenerating RLN or from the external branch of the SLN, but that collectively fail to produce electrical activity that can be measured with a surface recording electrode. Superior laryngeal nerve and RLN stimulation with intramuscular recording made from bipolar concentric needle electrodes could detect these motoneuron units.

In addition, recording of the RLN compound action potential (CAP) at a site proximal to the RLN's entry into the larynx might elucidate whether motoneuron axons have regenerated in the RLN. A stimulating electrode could be placed on the ipsilateral vagus nerve, and a recording electrode could be placed on the RLN just proximal to its entry into the larynx. After vagal stimulation, the absence of a recordable CAP would imply that motoneuron axons have failed to regenerate along the RLN between the stimulating and recording electrodes. This finding would confirm failure of the motoneuron axons to reach motor end plates, or true paralysis. Alternatively, the presence of a normal CAP would imply that although motoneuron axons have regenerated, they have not made functional connections to the motor end plate. The presence of a diminished CAP would imply incomplete regeneration of motoneuron axons.

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Ann Otol Rhinol Laryngol 110:2001

ONCOLOGICAL RESULTS OF ENDOSCOPIC RESECTIONS OF TIS AND T1 GLOTTIC CARCINOMAS BY CARBON DIOXIDE LASER

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A cohort of 88 patients with glottic cancer (13 Tis, 75 T1) who underwent endoscopic CO_2 laser excision between January 1995 and June 1997 was prospectively studied. The mean follow-up was 43 months (range, 30 to 60 months). The depth and extent of the excision (graded according to the European Laryngological Society Classification, which includes 5 types of resection) were based on the results of a preoperative and intraoperative diagnostic test battery. Five patients died of other diseases, and none of glottic cancer. Of the 12 patients who developed a local recurrence, 5 underwent a second endoscopic procedure, 5 a total laryngectomy, and 1 a supracricoid laryngectomy, and 1 was treated with radiotherapy. The 5-year local control rate with endoscopic surgery alone, according to the Kaplan-Meier method, was 91%. None of the variables (8 related to the tumor and 2 to the treatment) tested in a univariate analysis by the log-rank test was found to have a significant impact on disease-free survival rates. The present study confirmed that endoscopic partial cordectomy for Tis and T1 glottic cancers can be regarded as a valid alternative to radiotherapy in terms of oncological results.

KEY WORDS — carbon dioxide laser, conservative treatment, glottic cancer.

INTRODUCTION

Radiotherapy and open neck surgery (cordectomy, vertical partial laryngectomy) have been traditionally considered comparable therapeutic options for Tis and T1 glottic carcinomas, in that they provide equivalent long-term survival and local control. Data in the literature of the past 2 decades have produced convincing evidence that endoscopic surgery with the CO_2 laser can be considered a valid alternative,[1-9] resulting in a decreased popularity of open neck surgery. Furthermore, radiotherapy and endoscopic surgery have recently undergone significant technical improvements aimed at decreasing the complication rate and enhancing the quality of life. Treatment selection is based on a number of factors: patient counseling, functional outcome, medical facilities, cost-effectiveness ratio, and quality of the single technique.

As regards endoscopic surgery, the increased accuracy of the preoperative and intraoperative tools used to assess the extent and depth of the lesion and the development of more sophisticated instrumentation and operative techniques have made it possible to differentiate the extent of the resection. On the basis of this principle, several authors[3,4,9-13] have introduced different types of endoscopic excisions with the intent to respect the multilayered structure of the vocal cord and consequently to optimize the compromise between the extent of tissue resection and vocal function.

Since 1987, the concept of excisional biopsy, a 1-stage procedure allowing a histopathologic diagnosis and definitive treatment,[1,14] has been applied in our department in the management of selected glottic cancers. Between January 1987 and December 1994, 140 patients with previously untreated Tis, T1, and T2 glottic carcinomas underwent different types of CO_2 laser resection, graded according to a personal system of classification[9] that is conceptually equivalent to that recently introduced by the European Laryngological Society Working Committee (see Figure and Table 1).[13] In this group of patients, false vocal cord involvement and impairment of vocal cord mobility were found to have an unfavorable prognostic impact on the local control rate. Therefore, we currently restrict the indications for endoscopic surgery to Tis and T1 glottic carcinomas. According to the lastest version of the American Joint Committee on Cancer (AJCC)–Union Internationale Contre le Cancer (UICC) TNM classification of malignant tumors,[15,16] lesions extending to the floor of the ventricle have also been included in the latter category.

The aim of the present study was to prospectively validate the treatment guidelines on the basis of our previous experiences[9,14] with a cohort of patients affected by Tis and T1 glottic squamous cell carcinomas. For this purpose, the oncological results and the prognostic impact on disease-free survival of several variables related to the tumor and the treatment were analyzed.

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Presented at the meeting of the American Laryngological Association, Orlando, Florida, May 13-14, 2000.

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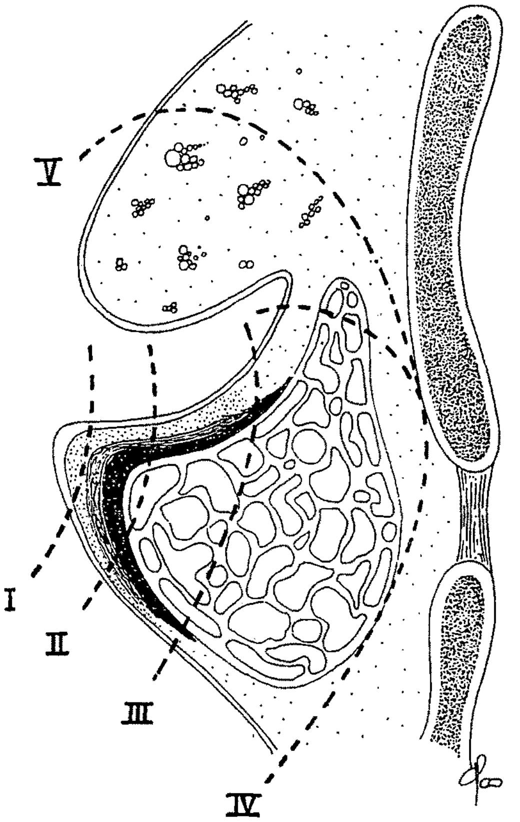

Drawing represents larynx in coronal plane with 5 types of resection. I — subepithelial cordectomy; II — subligamental cordectomy; III — transmuscular cordectomy; IV — total cordectomy; V — extended cordectomy.

MATERIALS AND METHODS

In the period from January 1995 to June 1997, 121 patients with clinical evidence of a glottic erythroleukoplakia underwent endoscopic CO2 laser excisional biopsy at the Department of Otolaryngology of the University of Brescia, Italy.

We excluded from the present analysis 33 patients in whom the definitive histopathologic diagnosis was keratosis without atypia or mild to moderate dysplasia. Therefore, the study focused on a cohort of 88 consecutive cases (13 Tis, 75 T1). The inclusion criteria encompassed patients with glottic carcinoma, eventually extending to the floor of the ventricle, without supraglottic or subglottic involvement or impairment of vocal cord mobility. Even though 20 cases with involvement of the floor of the ventricle were primarily included in the T2 category, they were subsequently reclassified as T1 according to the 1997 AJCC-UICC TNM classification.[15,16] None of the patients had received previous treatment. Twenty-seven of them had already undergone random biopsies for diagnostic purposes at another institution, and they came to our observation with a glottic erythroleukoplakia suggesting persistent disease. The population included 85 men and 3 women ranging in age from 38 to 86 years (mean, 61 years). Most of the patients were cigarette smokers; only 7 men and 1 woman had never smoked.

TABLE 1. PRIMARY TREATMENT METHOD (N = 88)

Type of Excision	*Extent of Excision*	*No. of Patients*
Subepithelial cordectomy (type I)	Superficial layer of lamina propria	10
Subligamental cordectomy (type II)	Superfical portion of vocalis muscle	17
Transmuscular cordectomy (type III)	Medial portion of vocalis muscle	27
Total cordectomy (type IV)	Inner perichondrium of thyroid lamina	21
Extended cordectomy (type V)	Surrounding laryngeal subsites	13

A wide exposure of the glottis by a Dedo or an Ossoff-Holinger laser laryngoscope with the Boston University suspension system (Pilling, Philadelphia, Pennsylvania) was necessary to select the patients for endoscopic treatment. We used a Sharplan 1055 S CO2 laser (Sharplan Lasers, Tel Aviv, Israel) set in the superpulse mode (1 to 3 W, 270-µm spot size).

Patients with a lesion involving the anterior commissure at clinical inspection underwent a preoperative computed tomography scan to rule out cartilage invasion. The deep and superficial extents of the lesion were routinely evaluated by a diagnostic test battery[9] including preoperative videolaryngostroboscopy; intraoperative rigid endoscopy by 0°, 30°, 70°, and 120° telescopes; supravital staining with 2% toluidine blue; and saline infusion in Reinke's space.

The results of the test battery led to different types of resection. Type I (subepithelial cordectomy) was used for a lesion preoperatively assumed not to extend beyond the basal membrane (ie, with evident intraoperative mucoligamentous hydrodissection and mucosal wave preservation at videolaryngostroboscopy). Type II (subligamental cordectomy) was applied in suspected microinvasive and invasive carcinoma (ie, with no mucoligamentous hydrodissection and an absent mucosal wave). Type III (transmuscular cordectomy) was used in cases of previous incisional biopsy. Type IV (total cordectomy) was used when the lesion extended to the anterior third of the vocal cord. Type V (extended cordectomy) was used in cases of anterior commissure and/or contralateral vocal cord involvement (Table 1).[9] Cancer resection was always performed by a 1-piece removal technique. Intraoperative frozen section examination of the surgical bed was never performed. For histologic evaluation, the surgical specimen was oriented by staining the superior edge with marking ink. Multiple sections were cut along the transverse axis, and the depth of

TABLE 2. UNIVARIATE SURVIVAL ANALYSIS FOR PROGNOSTIC FACTORS (LOG-RANK TEST)

Variable	*No. of Patients*	*Estimated 5-y Disease-Free Survival (% ± SE)*	*p*
Pathological T category			
Tis	13	92 ± 7	.6
T1	75	85 ± 4	
Involvement of anterior third of true vocal cord			
No	10	90 ± 9	.7
Yes	78	86 ± 4	
Involvement of middle third of true vocal cord			
No	17	94 ± 6	.3
Yes	71	84 ± 4	
Involvement of posterior third of true vocal cord			
No	60	85 ± 5	.6
Yes	28	89 ± 6	
Anterior commissure involvement			
No	76	87 ± 4	.7
Yes	12	83 ± 11	
Ventricle floor involvement			
No	68	88 ± 4	.3
Yes	20	80 ± 9	
Vocal ligament involvement			
No	18	89 ± 8	.7
Yes	70	86 ± 4	
Vocal muscle involvement			
No	86	86 ± 4	.6
Yes	2	*	
Neoplastic involvement of surgical margins			
No	44	84 ± 6	.6
Yes	44	89 ± 5	
Type of excision			
I	10	*	.09
II	17	82 ± 9	
III	27	96 ± 4	
IV	21	71 ± 10	
V	13	85 ± 10	

*Survival estimates could not be calculated, because no recurrence was observed.

infiltration was estimated.

All patients were subsequently examined every 2 months by laryngoscopy with a rigid 70° telescope or a flexible fiberoptic laryngoscope for a period ranging from 30 to 60 months (mean, 43 months). In 57 of them, a videolaryngostroboscopic examination was performed and the findings were graded according to a personal rating system (1, 2, 3). Score 1 corresponded to cases with variable degrees of glottic incompetence and an absent mucosal wave; score 2 to a condition of complete glottic closure with minimal impairment of mucosal wave progression; and score 3 to a normal pattern of vocal cord vibration. Laser reexcision was planned for patients with lesions involving the deep margin of the resection or with areas suspicious for persistent neoplasm at the first follow-up visit.

Statistical analysis of the data was performed with the SPSS statistical package. Survival curves were calculated from the date of the diagnosis by the Kaplan-Meier method. The end point for overall survival was the date of death (regardless of the cause) or the day of the last consultation for patients who were alive and well. Determinate survival could not be estimated, because no patient died of the laryngeal neoplasm. The end point for disease-free survival was the date of the first locoregional recurrence. For ultimate local control with laser alone, patients who had a relapse at the primary site and who were subsequently cured by further laser treatment were considered free of disease at the date of the last consultation.

The impact on the disease-free survival rate of 8 factors related to the tumor (pT category, localization of the tumor on the anterior, middle, and/or posterior thirds of the vocal cord, involvement of the anterior commissure and/or the floor of the ventricle, and histologic infiltration of the vocal ligament or muscle) and 2 related to the treatment (type of excision, positive margins) was evaluated by univariate analysis with the log-rank test (Table 2).

RESULTS

There have been no intraoperative or postoperative complications in any of the 88 patients; none of them required tracheotomy at the end of the procedure, and all were discharged the day after the operation. No swallowing difficulties were encountered by patients who underwent total or extended cordectomy.

All the patients who had undergone a previous biopsy elsewhere yielded surgical specimens that showed cancer (transmuscular cordectomy).

Twelve patients underwent a CO_2 laser reexcision (4 for positive deep margins of resection, and 8 for a suspicion of persistent neoplasm at the first follow-up visit); 3 cases showed residual foci of microinvasive carcinoma, and 1 case showed foci of carcinoma in situ. The remaining 8 patients did not present any histopathologic findings of persistent disease.

Twelve patients experienced local recurrence within periods varying from 4 to 26 months (mean, 13 months). One (8%) and 11 (15%) recurrences occurred, respectively, in the Tis and T1 groups. A second endoscopic procedure was performed in 5 patients, open neck surgery in 6 (5 total and 1 supra-

TABLE 3. ANALYSIS OF PATIENTS WHO DEVELOPED RECURRENCE

Pt. No.	*Initially Involved Structures (T Category)*	*Type of Excision*	*Site of Recurrence (Time in Months)*	*Treatment*	*Outcome (Months After Primary Treatment)*
1	Anterior, middle, and posterior thirds of TVC, vocal ligament (T1)	II	Homolateral TVC (14)	LE	A&W (60)
2	Middle third of TVC, ventricle floor, vocal ligament (T1)	IV	Homolateral paraglottic space with cricoarytenoid joint involvement (12)	TL	A&W (49)
3	Anterior and middle thirds of TVC, vocal ligament (T1)	IV	Anterior commissure, subglottic extension exceeding 10 mm (15)	TL + ND	A&W (47)
4	Anterior and middle thirds of TVC (Tis)	II	Homolateral TVC (8)	LE*	A&W (42)
5	Anterior third of TVC, vocal ligament (T1)	IV	Anterior commissure with extralaryngeal spread (20)	TL + ND	Died of lung cancer (29)
6	Anterior and middle thirds of TVC, vocal ligament (T1)	IV	Homolateral TVC (20)	RT	A&W (40)
7	Anterior and middle thirds of TVC, vocal ligament (T1)	II	Homolateral TVC (4)	LE	A&W (40)
8	Anterior and middle thirds of TVC, ventricle floor, vocal ligament (T1)	IV	Homolateral paraglottic space with cricoarytenoid joint involvement (17)	TL + ND	A&W (37)
9	Anterior and middle thirds of TVC, anterior commissure, ventricle floor, vocal ligament (T1)	V	Anterior commissure with extralaryngeal spread (11)	TL + ND	A&W (37)
10	Anterior, middle, and posterior thirds of TVC (T1)	III	Contralateral TVC (30)	LE	A&W (33)
11	Anterior and middle thirds of TVC, ventricle floor, vocal ligament (T1)	IV	Homolateral TVC (5)	LE	A&W (32)
12	Anterior, middle, and posterior thirds of TVC, anterior commissure, vocal ligament (T1)	V	Homolateral paraglottic space (11)	SCL + ND	A&W (31)

TVC — true vocal cord, LE — laser excision, A&W — alive and well, TL — total laryngectomy, ND — neck dissection, RT — radiotherapy, SCL — supracricoid laryngectomy.

*Had second recurrence in homolateral and contralateral TVCs 9 months later, treated by LE and RT.

cricoid laryngectomy), and radiotherapy in 1 (Table 3). The ultimate rate of laryngeal preservation was 94% (5 larynges sacrificed in 88 patients).

There have been 5 deaths of unrelated causes from 16 to 39 months after primary surgery: 2 patients died of a second pulmonary cancer, 2 of a stroke, and 1 of a myocardial infarction. No patient died of the laryngeal neoplasm.

The 5-year overall survival rate was 94% (92% for Tis, 94% for T1), the 5-year disease-free survival rate was 86% (92% for Tis, 85% for T1), and the ultimate rate of local control with endoscopic laser excision alone was 91% (92% for Tis, 90% for T1). Analysis of the impact of 10 categorical variables (pT category, localization of the tumor on the anterior, middle, and/or posterior thirds of the vocal cord, involvement of the anterior commissure and/or the floor of the ventricle, histologic infiltration of the vocal ligament or muscle, type of excision, and positive margins) on the disease-free survival rate of the patients failed to show significant differences on the log-rank test (Table 2). The probability of having a false-negative result on the log-rank test, because of the small sample size, was greater than .20.

As regards the stroboscopic findings, after subepithelial cordectomy (type I) and subligamental cordectomy (type II), 53%, 40%, and 7% of the patients had scores of 3, 2, and 1, respectively. After transmuscular cordectomy (type III), the same scores were respectively assigned to 24%, 57%, and 19% of the patients. Finally, 38%, 38%, and 24% of the patients treated by total (type IV) or extended cordectomy (type V) had scores of 3, 2, and 1, respectively.

DISCUSSION

Tis and T1 vocal cord lesions include a wide spectrum of histopathologic entities ranging from intraepithelial neoplasia to microinvasive and invasive carcinomas. Carcinoma in situ infiltrates the full thickness of the epithelium, but not the basal membrane. In contrast, microinvasive and invasive carcinomas penetrate the basal membrane and involve the lamina propria without deep vocal muscle infiltration.[17]

Even though the correct diagnosis of such histopathologic entities can be confirmed only on the surgical specimen, we routinely applied a preoperative and intraoperative test battery to minimize the number of clinical misdiagnoses.[9] By combining the results of intraoperative saline infusion into Reinke's space with videolaryngostroboscopic images, we obtained reliable information on the deep extent of the lesion beyond the basal membrane. In our hands, saline infusion and videolaryngostroboscopy have proved to be the most useful tools of the workup, with sensitivities of 83% and 100%, specificities of 90% and 82%, and accuracies of 86% and 91%, respectively (unpublished observations). Their combined use allowed us to select the most appropriate excision and thus to drastically reduce both undertreatment and overtreatment. In fact, as reported by other authors,[18,19] an absent mucosal wave or impairment of its progression along the vocal surface correlated well with an incomplete mucoligamentous hydrodissection, thereby suggesting infiltration of the lamina propria. The multiperspective view obtained with 0°, 30°, 70°, and 120° rigid endoscopes[20] and supravital staining with 2% toluidine blue[21] allowed a more accurate evaluation of the superficial extent of the lesion and therefore decreased the incidence of positive surgical margins. Furthermore, the supravital staining technique was helpful in identifying areas of second primary dysplasia or cancer.

Therefore, subepithelial cordectomy (type I) and subligamental cordectomy (type II) should be considered oncologically sound resections, respectively, for Tis and T1 carcinomas. In fact, in the present cohort, only 2 patients of 75 with T1 lesions (2.6%) showed a superficial vocal muscle infiltration at histopathologic examination. Nevertheless, we performed a transmuscular cordectomy (type III) in 27 patients who had previous vocal cord random biopsies performed at another center, an event that could have led to alterations of the multilayered vocal cord structure (ie, fibrosis or granulomas), which can make the results of the preoperative assessment unreliable. According to the conclusions of our previous retrospective study,[9] we consider total cordectomy (type IV) the elective procedure for lesions involving the anterior third of the vocal cord, because of the high recurrence rate observed in such localized tumors when treated by more conservative resections. Finally, we reserve extended cordectomy (type V) for lesions spreading to the anterior commissure and/or to the anterior third of the contralateral vocal fold. The validity of the aforementioned criteria in selecting the type of cordectomy to perform in Tis and T1 glottic carcinomas is confirmed by the present oncological results, which compare favorably with the cure rates of 80% to 98% reported in the literature regardless of the treatment method.[1,2,6,22-26]

Our results confirm the prognostic validity of the modification introduced in the last AJCC-UICC TNM classification, which regards the floor of the ventricle as a glottic subsite. In fact, the invasion of the floor of the ventricle observed in 20 patients in our series did not show any significant impact on the disease-free survival rate (80% versus 88%; $p = .3$). Moreover, our data regarding disease-related deaths and laryngeal preservation (94%) favorably correlate with the results obtained with radiotherapy and surgery.[25] Accordingly, endoscopic surgery should be regarded as an effective treatment method even for recurrent vocal cord carcinomas, as supported by our series, in which ultimate local control was obtained with the laser alone in 5 of 12 recurrences (42%).

Endoscopic treatment of glottic tumors involving the anterior commissure has been classically controversial. In spite of a negative impact of anterior commissure invasion on the cure rate for stage I glottic carcinoma reported by a number of investigators,[3,22,23,27-29] endoscopic resection of early glottic cancer involving the anterior commissure has been performed successfully by others.[4,6,9,30-32] In our experience, effective surgery in this region is optimized by an adequately designed laryngoscope, by having the patient placed in the Boyce-Jackson position, and by the multiperspective view of the lesion offered by 0°, 30°, 70°, and 120° telescopes.[33] Moreover, as suggested by Zeitels,[33] resection of the anterior portion of both false vocal cords and the infrapetiole region should be routinely performed to provide clear visualization of the superior surface of the anterior commissure and to assess the neoplastic invasion of the underlying cartilage framework. Kirchner[34] showed with whole organ sections that neoplasms confined to the level of the glottis with mobile vocal cords are not deeply invasive and do not penetrate the underlying thyroid cartilage, which is protected by the fibroelastic tissue of Broyle's ligament. In contrast, anterior glottic lesions spreading to the base of the epiglottis and/or the subglottis invade the thyroid cartilage in almost every case.

On the basis of these observations, well supported by our data confirming that anterior commissure localization (12/88) did not negatively affect primary local control (83% versus 87%; $p = .7$), we recommend that endoscopic treatment be limited to selected cases of anterior commissure cancer superficially spreading at the level of the glottis without extension to the supraglottis or subglottis.

The debate concerning vocal function after laser or conventional surgery or radiotherapy for glottic carcinoma is still open. Conflicting results are mostly related to the difficulty of comparing different techniques of cordectomy with radiotherapy, to the criteria used for patient selection, and to the method of vocal quality evaluation. Better voice quality after laser cordectomy than after conventional cordectomy has been reported.[35,36] This could be explained by the possibility that the endoscopic procedure is associated with a lower incidence of anterior synechiae. Radiotherapy and endoscopic resection may both produce an abnormal voice, the former causing edema, stiffness, scarring, atrophy, and mucosal changes of the entire glottis, and the latter producing scarring of the operated vocal cord or even glottic incompetence in relation to the depth of excision. Some authors have reported that the voice is significantly better after radiotherapy,[37,38] whereas others observed equivalent vocal quality in patients with laser treatment and patients with radiotherapy.[11,39,40] The latter authors maintained that preservation of the anterior commissure and more than half of the vocal muscle plays a key role in the functional outcome.

Our postoperative stroboscopic examinations are in keeping with such observations. We found a correlation between the postoperative functional outcome and the amount of tissue and anatomic structures removed (ie, anterior commissure, vocal muscle, and part of the contralateral vocal cord). In fact, 93% of the patients who underwent type I or type II cordectomy had complete closure of the glottis (scores 3 and 2), and 53% of them displayed a normal pattern of vibration (score 3), whereas 24% of patients who had type IV or type V resections showed incomplete closure and a frequently absent mucosal wave (score 1). Therefore, careful patient selection is the key to achieving a favorable vocal outcome after endoscopic resection.

A variety of other advantages of endoscopic surgery over radiotherapy, such as a favorable cost-effectiveness ratio, less morbidity, a low complication rate, and no preclusion of further treatments (surgery or radiotherapy), have been already addressed by several authors.[19,41,42]

In conclusion, the results of the present study support the validity of CO2 laser excision in the treatment of Tis and T1 glottic cancers. The routine application of a series of preoperative and intraoperative tests accurately assessed the depth and the superficial spread of the lesion and allowed us to adjust the extent of the resection according to a personal system of classification. Objective evaluation of the voice following the different endoscopic resections is under evaluation in our institution; data generated by the analysis will certainly permit us to further refine our strategy of treatment for Tis and T1 glottic cancers.

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LARYNGEAL MASK VERSUS ENDOTRACHEAL TUBE IN A FERRET MODEL

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Acquired subglottic stenosis in infants is a difficult iatrogenic problem with notable morbidity, primarily caused by prolonged endotracheal intubation. The laryngeal mask airway (LMA) is a recently developed, alternative airway device that does not contact the subglottis. To explore the possibility of preventing subglottic stenosis, we compared the endotracheal tube (ETT) and the LMA in terms of the incidence and severity of glottic and subglottic injury resulting from prolonged intubation in the adult ferret model of the infant airway. Ten adult ferrets were randomly intubated under inhalational anesthesia with either a 4.0 cuffless ETT or a size 1 LMA for a 24- to 48-hour period. Rigid laryngeal endoscopy was used to detect pharyngeal or glottic injury during the period of intubation and on a routine basis for 3 months after extubation. All 5 ferrets in the ETT group developed endoscopically evident glottic and subglottic injury; 2 of the 5 developed a symptomatic, mature subglottic stenosis. The 5 ferrets in the LMA group had endoscopically normal larynges. However, all ferrets in the LMA group developed significant tongue edema and cyanosis during the first 24 hours of intubation, and 3 of the 5 died of respiratory failure due to airway obstruction. In the 2 LMA survivors, evidence of oropharyngeal injury persisted until 6 weeks after extubation. We conclude that the LMA does not cause subglottic injury in this model. However, its prolonged use results in significant pharyngeal morbidity that raises serious doubt as to its potential routine use in infants requiring prolonged ventilatory support.

KEY WORDS — endotracheal tube, laryngeal mask airway, subglottic stenosis.

INTRODUCTION

Infant resuscitation is an increasingly frequent occurrence. More than 6% of the approximately 3.5 million neonates born each year in the United States require advanced life support, often with endotracheal intubation,[1] and 80% of infants weighing less than 1,500 g require advanced life support. Acquired subglottic stenosis is a known complication of endotracheal intubation, with a current incidence ranging from 1% to 9%.[2,3] Unfortunately, the current medical and surgical treatments for subglottic stenosis have significant rates of morbidity and mortality.

The subglottic injury model of subglottic stenosis has been well described.[4] Although the causes of subglottic stenosis are likely varied, clearly, the presence of prolonged subglottic instrumentation with an endotracheal tube (ETT) is the primary inciting factor. The laryngeal mask airway (LMA) is a relatively new, alternative airway device that secures the airway without subglottic or tracheal instrumentation.[5] Its efficacy and safety are well documented in the pediatric and neonatal populations.[6-11] However, its use for prolonged periods (24 hours or greater) of respiratory support in this population has not been described. Could the LMA be used in place of the ETT for prolonged ventilatory support with a reduced or eliminated incidence of subglottic stenosis? Concerns about prolonged LMA intubation that have precluded its use thus far include aspiration risk, pharyngeal morbidity, and lack of definitive airway control.[12]

The adult ferret is an excellent model of the infant airway (Fig 1). Many studies have successfully used the ferret in the examination of pediatric subglottic pathophysiology.[13,14] It has proved to be a reliable model of laryngeal injury subglottic stenosis. Previous investigators have produced subglottic stenosis both by direct laryngeal injury (wire brush), and by modified endotracheal intubation.[15,16] Ferrets are also hardy, inexpensive animals with an extensive, well-described role in research.

The purpose of this study was to use the ferret model to compare the ETT and the LMA in terms of the incidence and nature of pharyngeal and subglottic injuries produced during prolonged ventilatory support (24 to 48 hours). This was done in an envi-

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From the Department of Otolaryngology–Head and Neck Surgery, Walter Reed Army Medical Center, Washington, DC. The opinions or assertions of the authors contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Armed Forces Radiobiologic Research Institute.

Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

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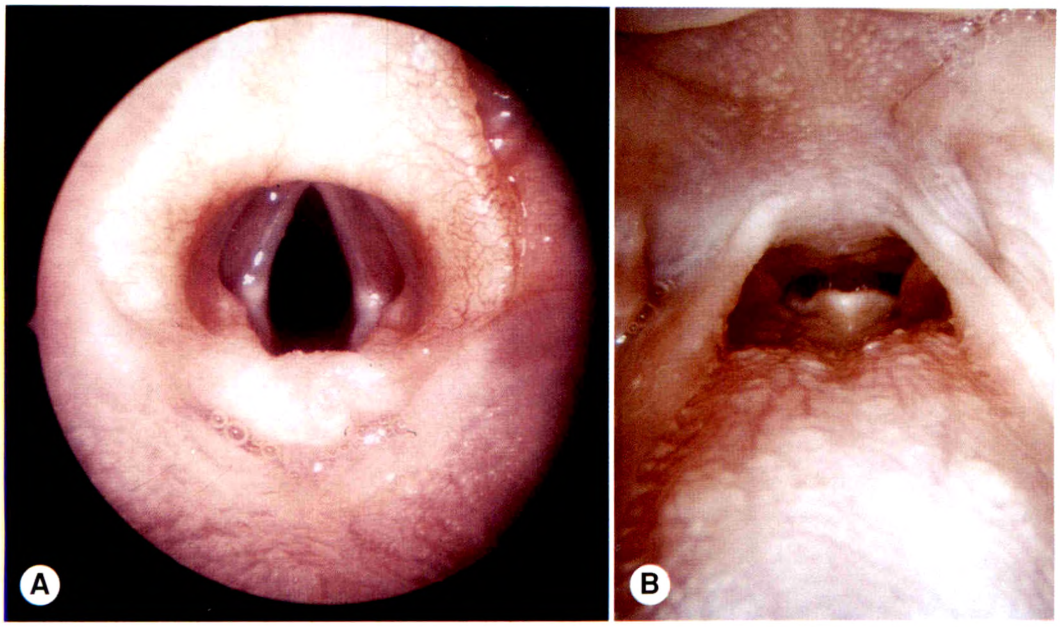

Fig 1. A) Normal ferret glottis. **B)** Normal ferret oropharynx.

ronment closely approximating the neonatal intensive care unit (ICU) with continuous 24-hour sedation and anesthesia and continuous monitoring.

MATERIALS AND METHODS

This animal protocol was approved by the Institutional Animal Care and Use Committee and was performed in accordance with all institutional guidelines regarding animal experimentation.

Ten adult male ferrets (Marshall Farms, North Rose, New York) weighing 1.6 to 2.5 kg were used. After an appropriate acclimatization period, relaxation was induced with intramuscular ketamine hydrochloride, and topical laryngeal anesthesia (2% lidocaine hydrochloride) was administered. Each animal then underwent a baseline rigid telescopic examination (Karl Storz Endoscopy–America Inc, Culver City, California) of the larynx and trachea with photodocumentation. It was then randomly intubated with either a 4.0 cuffless ETT (5 ferrets) or a No. 1 LMA (5 ferrets). Endotracheal intubation was performed by standard direct laryngoscopy techniques. Leak pressures were less than 20 cm H_2O. The LMA was modified before placement by removing the mask airway bars to allow easy passage of a flexible endoscope for evaluation of the airway while leaving the tube in place (Fig 2). This modification has been commonly performed at our institution for years to enhance fiberoptic visualization in pediatric patients and does not affect the function or safety of the device. The LMA was then positioned in the airway by the standard technique, and the cuff was inflated with 1 to 3 mL of air until there was no audible leak, as described by the manufacturer.[17] Correct tube placement for both groups was confirmed with auscultation and observation of tube misting. Intraperitoneal or intravenous catheters were then placed for medication administration and hydration.

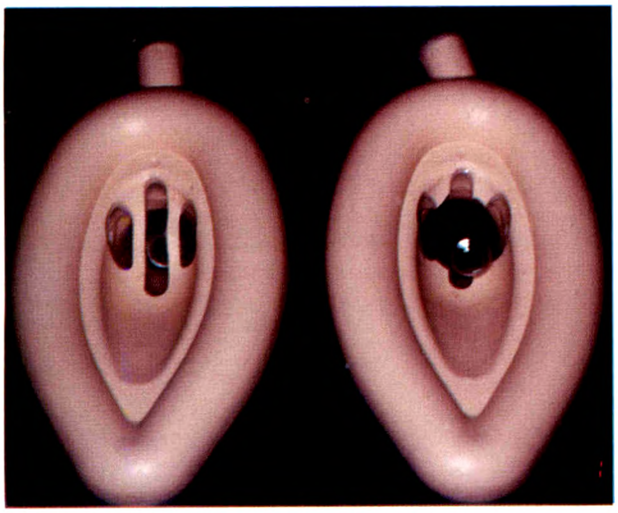

Fig 2. Size 1 laryngeal mask airway (LMA) modified by removal of mask airway bars to facilitate airway suctioning and fiberoptic visualization. This modification does not affect function or safety of LMA and is commonly performed in pediatric LMA intubations at our institution.

Animal monitoring and care was performed to an ICU standard. The "ferret ICU" was staffed by 2 or more trained personnel 24 hours a day. Continuous anesthesia and sedation was provided by inhaled isoflurane. The animals were not mechanically ventilated, but were given supplemental oxygen and allowed to spontaneously ventilate. Routine animal care included continuous vital sign monitoring, scheduled animal position rotation, pulmonary toilet, eye care, intermittent positive pressure ventilation, and intra-

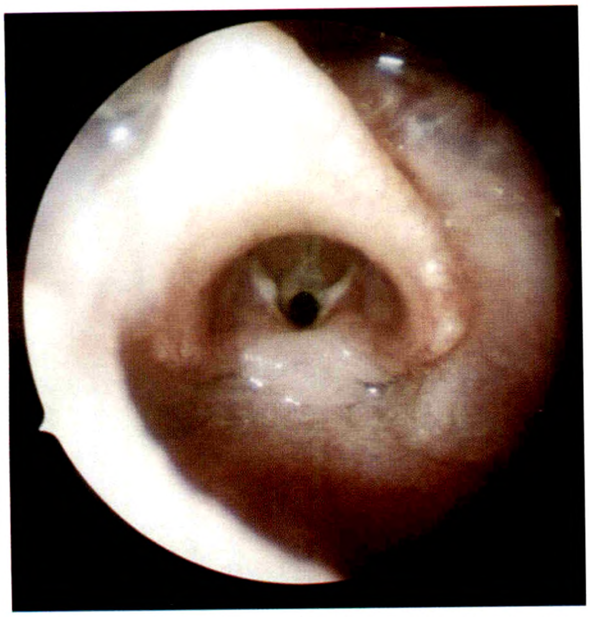

Fig 3. Ferret larynx and subglottis immediately after removal of endotracheal tube (ETT) after 24 hours of intubation. Note posterior glottic edema and erythema and "stenting" of vocal folds to accommodate ETT.

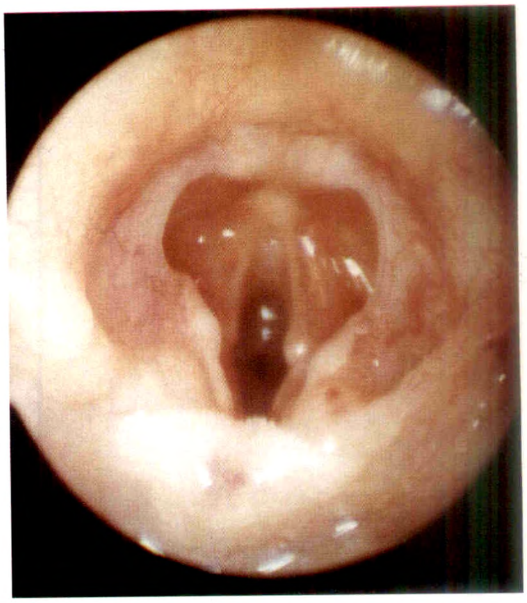

Fig 4. Ferret larynx and subglottis 3 weeks after extubation with ETT. Note severe subglottic stenosis with "pinhole" airway. Animal was symptomatic and was euthanized.

venous or peritoneal hydration. The planned duration of intubation was 24 to 48 hours. Initial pilot studies had included planned enteral tube feeds for nutrition. Unexpected problems occurred in the form of reduced gastric motility with gross emesis and aspiration. Because of the relatively brief duration of intubation, parenteral or enteral nutrition was subsequently not provided to the animals. After the planned period of intubation and sedation, all animals were recovered from anesthesia, advanced to a regular diet, and returned to the colony. No antibiotics or medications of any kind were administered after intubation. All animals that died during the study underwent full necropsy evaluation.

All ferrets underwent rigid endoscopic evaluation every 24 hours while intubated. After extubation, all animals underwent weekly endoscopic evaluations, during which they breathed spontaneously under sedation with ketamine and with topical laryngeal lidocaine.

Statistical analysis was performed with Fisher's exact test.

RESULTS

The animals randomized to the ETT group underwent either 24 hours (2 ferrets) or 48 hours (3 ferrets) of anesthetized intubation. Immediately upon extubation, endoscopic evaluation revealed posterior glottic and subglottic edema (Fig 3). The animals then underwent weekly endoscopic evaluations. At both the 3- and 4-week points, 1 ferret was found to be clinically stridorous, with apparent respiratory distress. Endoscopy revealed severe subglottic stenosis (Fig 4). Necropsy revealed a mature, concentric subglottic stenosis with squamous metaplasia of the tracheal epithelium. In 2 of the remaining 3 animals, less severe subglottic stenosis was observed upon weekly examinations and persisted at the conclusion of the study, 12 weeks after extubation. No endoscopically significant differences in the severity of the subglottic changes were noted between the 24-hour group and the 48-hour group. No other oropharyngeal morbidity was noted in the ETT group.

All animals randomized to the LMA group (5 ferrets) underwent approximately 24 hours of intubation. No animals were intubated more than 24 hours, because of the severity of observed oropharyngeal morbidity. No significant clinical changes were noted in respiratory signs or tongue color or size within the first 4 hours of LMA intubation. Four hours is the typical maximum duration of LMA intubation during routine outpatient surgery. All animals with the LMA in place began to develop clinically evident, progressive tongue edema and cyanosis after several more (6 to 16) hours of intubation (Fig 5). This phenomenon peaked at approximately 24 hours, and 3 of the 5 animals were euthanized because of respiratory failure due to airway obstruction (Fig 6). Reducing cuff pressures and repositioning the LMA had no apparent effect in reducing this swelling. Necropsy revealed massive swelling and venous congestion of the tongue. Histologic analysis of the tongue revealed stromal edema with capillary congestion and epithelial breakdown of the tongue surface in contact with the LMA (Fig 5). No comparable tongue or pharyngeal swelling was observed in the ETT group.

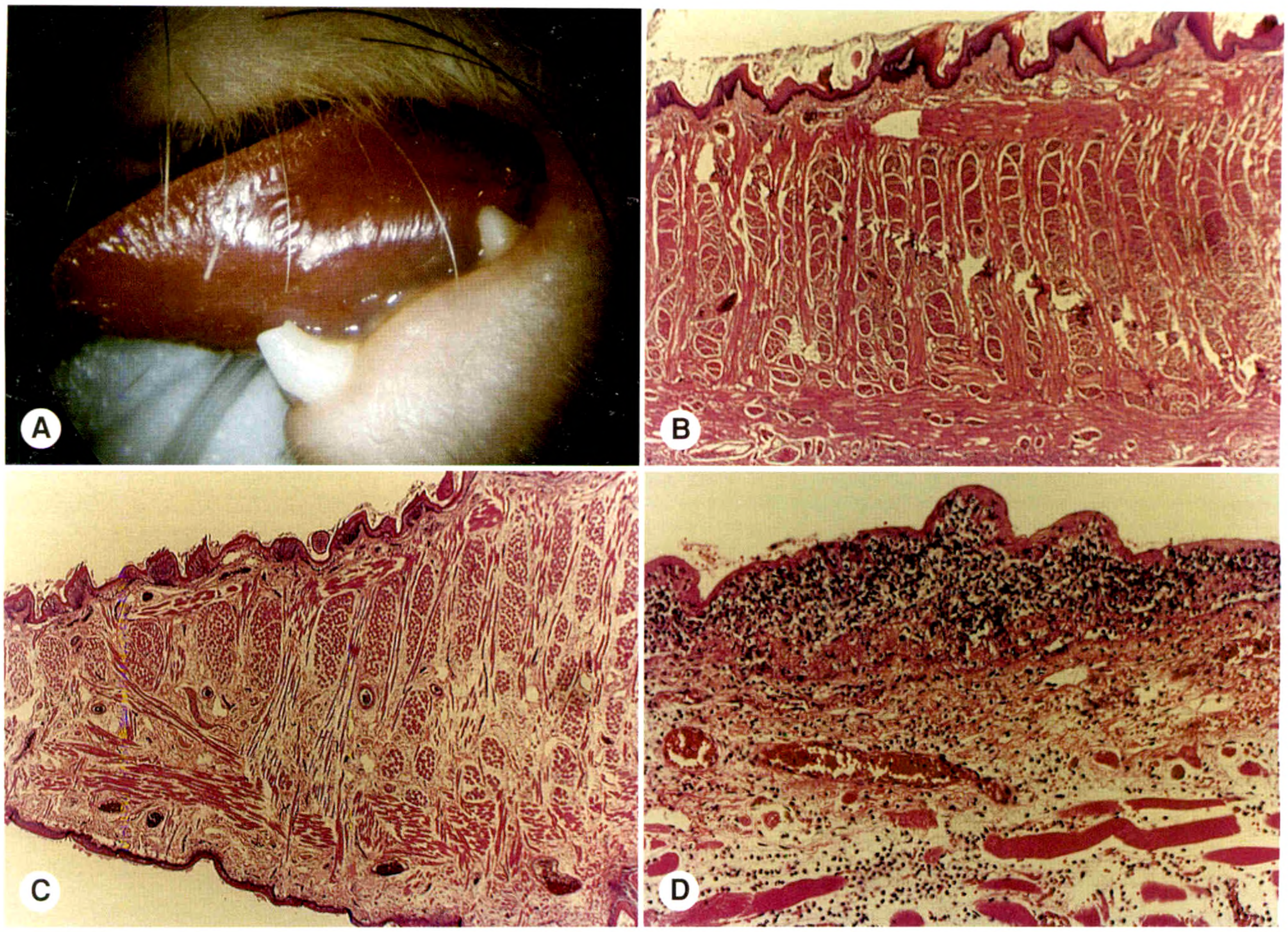

Fig 5. A) Ferret tongue after approximately 18 hours of LMA intubation. Note edema and prominent venous congestion. **B)** Photomicrograph of normal ferret tongue for comparison (H & E, original ×7.6). **C)** Photomicrograph of tongue in LMA group ferret euthanized for airway obstruction after 24 hours of LMA intubation (H & E, original ×7.6). Compare with B, and note severe stromal edema and acute inflammation with splaying of muscle fibers. **D)** Higher-power photomicrograph of same ferret tongue as in C, demonstrating intense acute inflammation and breakdown of epithelial surface in contact with LMA (H & E, original ×56).

This difference was found to be statistically significant ($p < .05$).

After extubation, weekly endoscopic evaluations of the surviving LMA animals demonstrated slow resolution of the oropharyngeal trauma. Persistent tongue granulation tissue and swelling were observed up to 6 weeks after extubation in 1 animal (Fig 7). No glottic or subglottic injury was noted in any LMA animal. The surviving animals were found to have no clinical respiratory distress or difficulties with oral intake.

Pulmonary toilet, of vital concern in prolonged ventilatory support, proved to be difficult in the LMA group. Tenacious, obstructing secretions were observed and contributed to the observed airway obstruction and subsequent respiratory failure (Fig 8). Even with the LMA modified by removal of the mask bars, it was very difficult to pass a suction catheter through the LMA and into the airway. When this was accomplished, the complications of laryngospasm and/or vocal cord damage had to be considered. Sometimes, the only effective way to achieve adequate pulmonary toilet and restore functional respiration was to extubate the animal, suction the larynx directly, and replace the LMA. This is clearly unacceptable from a practical standpoint and could not be a routine practice in the ICU.

DISCUSSION

The LMA has well-documented efficacy and safety in the short-term ventilation of infants. Concerns over long-term (>24 hours) use include oropharyngeal morbidity and lack of definitive airway control. Support for both of these concerns was clearly evident in this animal model study.

Cases of LMA-associated oropharyngeal trauma are uncommon, but have been reported in the literature. Reported events include trauma to the epiglottis,[18] uvula,[19] tonsils,[20] and posterior pharyngeal wall[21]; tongue cyanosis[22]; and lingual,[23] hypoglossal,[24] and recurrent laryngeal nerve palsies.[25] These

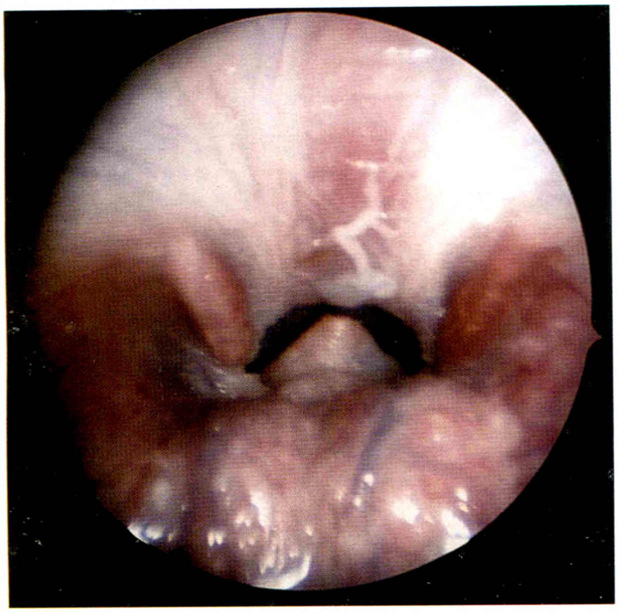

Fig 6. Ferret oropharynx immediately after 24 hours of LMA intubation. Note tongue edema and cyanosis, and erythema and mucosal injury of lateral pharyngeal walls. Larynx was free of injury.

reported adverse events are distinctly different from the oropharyngeal morbidity observed in this study. Nearly all were noted during very brief surgical cases (4 hours), and the majority could be attributed to improper insertion of the LMA, an LMA of incorrect size, excessive cuff pressure, or other extraneous factors (eg, prone positioning or patient anticoagulation).

We found only 1 report of LMA-induced tongue cyanosis,[22] despite its being the primary morbidity observed in our model. In the reported case, changes in the tongue appeared very soon after intubation and resolved with extubation and placement of an LMA that was 1 size smaller. Hence, the authors postulated that the likely cause of the cyanosis was compression of the lingual artery. In our prolonged-ventilation ferret model, the tongue edema was of gradual onset, becoming noteworthy only after 6 hours of LMA intubation, and it progressed with the increasing duration of intubation. It did not respond to changes in LMA position or cuff pressure. Unfortunately, use of a smaller LMA was not an option, as size 1 is the smallest size currently available and is the recommended neonatal size. Although the ferret larynx is quite similar to the infant larynx, there are some notable differences. The ferret possesses a larger tongue, a triangular, broad-based epiglottis, and redundant tissue in the posterior glottis. Despite these differences, the placement and fit of the size 1 LMA in the ferret oropharynx were thought to be appropriate. Fiberoptic endoscopic evaluation was performed in the early phases of the model development and confirmed the proper supraglottic placement of the size 1 LMA in the ferret oropharynx and hypopharynx. Using standard pediatric insertion techniques and the cuff inflation pressure recommended by the manufacturer, we were able to easily seat the size 1 LMA in the adult ferret oropharynx and hypopharynx in a fashion that was nearly identical to its use in the human infant and that permitted effective ventilation. Hence, it is unlikely that the morbidity observed with prolonged LMA use in this model was attributable only to the anatomic differences.

Compression of the venous drainage of the tongue

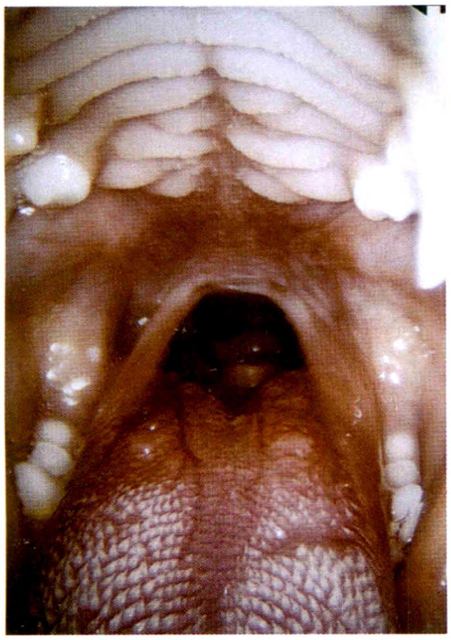

Fig 7. Ferret oropharynx 3 weeks after 24 hours of LMA intubation. Note persistent granulation tissue on surface of tongue. Pharyngeal injury has resolved. Animal was not symptomatic.

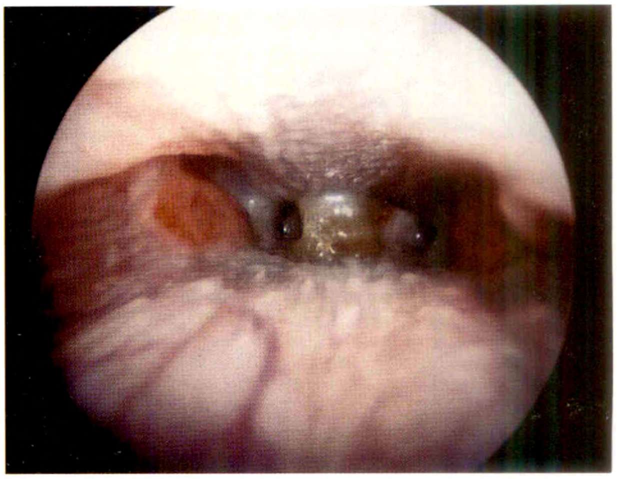

Fig 8. Ferret larynx after several hours of LMA intubation, demonstrating tenacious secretions nearly obstructing airway. Repeated attempts at passing suction catheter through LMA failed to remove secretions, and LMA has been momentarily removed to allow direct suctioning of airway.

was likely the cause of the tongue changes noted with prolonged LMA intubation in this animal model. It has been demonstrated that even at the recommended cuff pressure, the pressure applied to the pharyngeal mucosa by the LMA exceeds the capillary perfusion pressure.[26,27] As the duration of this applied pressure progresses, changes in the mucosa would be expected: first, edema and venous congestion (as we observed in the ferret tongues; Fig 5), and then, tissue necrosis. Histologic analysis of the tongues of the euthanized LMA ferrets confirmed the presence of severe venous congestion with early necrosis and epithelial breakdown. This sequence of events would be analogous to the well-documented effects of the ETT cuff on the tracheal mucosa and the resulting tracheal injury. The results of this animal model study raise concerns over the LMA's causing a similar injury pattern in the oropharynx.

The LMA has clearly been established as an integral part of the difficult airway algorithm for infants.[6,8,10,11,28] However, its lack of "definitive airway control" often relegates its role to that of a rapid airway-securing device to be used while a more definitive (ETT or tracheostomy) airway is established. Often, the LMA is used as a direct conduit for endoscopic-guided endotracheal intubation or flexible bronchoscopy. This study serves to highlight the presumed shortcoming of the LMA for "definitive" airway control in the case of prolonged ventilation. Pulmonary toilet was extremely difficult. Adequate suctioning was not possible through the infant-sized LMA. Although the LMA has a larger lumen than the ETT, it is actually more difficult to effectively suction the trachea and subglottis through it. Inspissated mucus builds up between the subglottis and the LMA cuff. The suction catheter is inserted blindly down the LMA and will typically not pass beyond the mask, through the glottis, and into the subglottis and trachea unless aided by direct visualization. Thus, to achieve adequate pulmonary toilet, we occasionally had to extubate the animal for direct suctioning of severe mucus plugging of the glottis and trachea and then reintubate the animal (Fig 8). This practice is certainly not feasible in the ICU setting, and repeated LMA intubation and extubation could further contribute to oropharyngeal morbidity.

As expected, no subglottic stenosis was observed in the LMA group, in contrast to the ETT controls. This finding was anticipated because of the lack of subglottic contact with the LMA. However, this benefit does not necessarily outweigh the significant problems of oropharyngeal morbidity, lack of definitive airway control, and problematic pulmonary toilet that were observed in this animal model. The LMA has a well-established role in pediatric short-term ventilation and resuscitation, but given the observations of this study, its prolonged use beyond 24 hours would require extreme caution and careful monitoring.

ACKNOWLEDGMENTS — The authors thank the tireless staff of the "ferret intensive care unit" for their hard work and dedication to this project: Jeff Greenwald, MD, Collen Lennard, MD, Allen Mitchell, MD, Ife Sofola, MD, Kelvin Buchanan, DVM, Tom Eggleston, DVM, Dean Hommer, MD, Phillip Littlefield, MD, Christine Barnacle, Chad Holmes, Matthew Marcuson, and Douglas White. We also thank Robin Howard, MA, for assistance with study design and statistical analysis and Keith Kaplan, MD, for assistance with histology.

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Ann Otol Rhinol Laryngol 110:2001

ANGIOTENSIN-CONVERTING ENZYME INHIBITOR–INDUCED ANGIOEDEMA: A MULTICENTER REVIEW AND AN ALGORITHM FOR AIRWAY MANAGEMENT

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Angioedema is a nonpitting edema of which the presentation ranges from benign facial swelling to airway obstruction managed by intubation or tracheotomy. The presentation of this disease is reviewed, and a treatment algorithm based on initial signs and symptoms is proposed for proper airway management. We performed a retrospective review of 108 patients treated in 2 tertiary care centers in the Washington, DC, area over a 5-year period. Ninety-eight patients (90.7%) were African-American, and 81 (75%) were female. Seventy-four patients (68.5%) were taking angiotensin-converting enzyme inhibitors (ACEIs). A classification system was developed based on the location of the edema at initial presentation: 1) isolated facial swelling and oral cavity edema, excluding the floor of the mouth; 2) floor of mouth and/or oropharyngeal edema, and 3) oropharyngeal edema with glottic and/or supraglottic involvement. Fourteen patients (13%) needed airway intervention, 2 of whom underwent a cricothyrotomy after a failed intubation attempt. Eleven (78.6%) were taking ACEIs. The indication for each intubation was massive tongue and floor of mouth edema. The patients were extubated 48 to 72 hours later. No patient demonstrated symptom progression after medical treatment was initiated. Therapy included discontinuation of the ACEI or other inciting agent, a high-humidity face tent, an initial dose of intravenous antihistamines, and a continued course of intravenous steroids. Within 48 hours, most patients had a resolution of their edema. Only cases of significant tongue and oropharyngeal edema took longer than 48 hours to resolve. The ACEIs are a common cause of angioedema. Left untreated, angioedema may progress to involve the oropharynx and supraglottis, resulting in a life-threatening airway compromise. Marked floor of mouth and tongue edema are the indications for airway intervention. An algorithm based on the initial presentation is essential for proper airway and patient management. Once treatment has begun, angioedema is nonprogressive and often resolves within 24 to 48 hours.

KEY WORDS — airway obstruction, angioedema, angiotensin-converting enzyme inhibitors.

INTRODUCTION

Angioedema is a nonpitting edema that occurs in skin and mucous membranes. It often initially presents in the head and neck as facial swelling, but can progress to major tongue and supraglottic edema resulting in airway compromise. Patients may present to emergency room physicians, internists, and otolaryngologists, by whom prompt recognition of the angioedema and institution of therapy is essential for proper airway management.

There are a multitude of precipitating factors, such as a hereditary deficiency of C1 esterase inhibitors, temperature extremes, trauma, food sensitivity, food additives, and medications such as aspirin and penicillin.[1] Angioedema has been recognized as a rare complication of angiotensin converting-enzyme inhibitors (ACEIs) for nearly 15 years. Earlier series on angioedema recognized ACEI use as a cause in 25% to 38% of cases.[2-5] With ACEI use steadily increasing secondary to better tolerance rates and fewer reported side effects,[6] ACEI-induced angioedema now accounts for a larger proportion of angioedema cases.

We reviewed our experience at 2 tertiary care referral centers over a 7-year period. Our goals were to 1) study the prevalence and demographics of ACEI-induced angioedema; 2) evaluate the differences between ACEI-induced and other types of angioedema; 3) investigate the clinical outcome and lack of disease progression once treatment was initiated; 4) compare the initial presentation and demographics in those who required airway intervention versus those who were only treated medically; and 5) use these data to develop a treatment algorithm for proper airway management based on initial patient signs.

METHODS AND MATERIALS

A retrospective chart review was performed on all patients carrying a DRG (diagnosis related group) code of angioedema at 2 tertiary care centers, the

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Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 15-16, 2000.

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Washington Hospital Center and Georgetown University Medical Center, for the years 1993 to 1999. These patients included those seen in the emergency department and those admitted for further management.

Demographic data, time from onset of symptoms to presentation, number of prior events, significant co-morbidities, and medications were recorded. We considered ACEIs to be the causative agent when angioedema occurred in patients taking these drugs in the absence of another likely agent. The specific ACEI used and the length of time it was used were recorded when available. Particular attention was paid to patient symptoms and initial presenting signs. Vital signs, presence of stridor, and areas of edema were noted, as well as findings made on direct laryngoscopy when it was performed at initial presentation. Care was taken to note the anatomic regions involved, specifically, the anterior tongue, floor of the mouth, oropharynx (defined as base of the tongue, uvula, and soft palate), supraglottis (defined as epiglottis, posterior pharyngeal wall, false vocal folds, arytenoids, and aryepiglottic folds), and glottis. Airway intervention was defined as intubation, cricothyrotomy, or tracheotomy.

The hospital course was reviewed for those patients admitted to the wards and intensive care units (ICUs). Methods of observation and medical treatment were recorded. Any evidence of symptoms or signs of progression were also noted. Significantly noticeable decreases in the area of edema on physical examination and on repeat fiberoptic laryngoscopy were considered to denote improvement.

The statistical analysis of data was performed with a χ^2 test for trend and Fisher's exact test. We considered p values of less than .05 to indicate statistical significance.

RESULTS

A total of 108 patients from the 2 institutions were reviewed. The average patient age was 60.9 years (median, 65.5; range, 20 to 92 years). Eighty-one patients (75%) were female and 27 (25%) were male. Ninety-eight patients (90.7%) were African-American, 7 (6.5%) were white, and the remaining 3 were Asian or Hispanic. Thirty-one patients (28.7%) reported having had a prior episode of angioedema. Given the high rate of ACEI users in this study, hypertension was the prevailing co-morbidity. Other common co-morbidities included asthma, diabetes, coronary artery disease, congestive heart failure, and seizure disorders. Two patients were former heart transplant patients currently taking immunosuppressants.

Patients taking an ACEI at presentation were presumed to have the drug as the causative agent of their angioedema if there were no other likely causes, ie, trauma, food allergies, or other medications such as penicillin. Accordingly, ACEI-induced angioedema was identified as the cause in 74 patients (68.5%). Table 1 compares the demographics and time until improvement. It also includes the location of treatment, ie, whether patients were discharged from the emergency room, admitted to the ward, or admitted to the ICU. The patients were African-American in 95.9% of the ACEI-induced cases, and in only 79.4% of the cases due to other acquired causes ($p < .05$). There were no statistical differences between patients with ACEI-induced episodes and the patients in each of the other acquired-cause groups in terms of sex, initial physical signs, time until improvement, or place of treatment.

Serum complement assays and assays for C1-esterase inhibitor were not routinely performed, and in those patients tested, not a single case of hereditary angioedema was identified by serologic studies of C1 esterase inhibitor levels or clinical history. The remaining cases not caused by an ACEI were attributed to sporadic causes.

There was a large range in the time elapsed from the initial onset of symptoms to presentation (30 minutes to 3 days; mean, 10 hours; median, 5 hours). Initial complaints were grouped into 3 categories: 1) facial swelling (41.7%), 2) swollen tongue and/or dysphagia (46.3%), and 3) shortness of breath and/or voice changes (16.7%).

Cases were classified into 3 categories based on the initial physical examination. Angioedema limited to the face and oral cavity, but excluding the floor of the mouth, was classified as type 1. Sixty-two patients (57.4%) fit this description. Type 2 angioedema (n = 28; 25.9%) was edema of the floor of the mouth and/or oropharynx, including the base of the tongue, soft palate, and uvula. Type 3 angioedema (n = 18; 16.7%) was oropharyngeal involvement with extension to supraglottic and glottic structures. There were no patients with isolated supraglottic or glottic involvement without accompanying oropharyngeal involvement.

Table 2 shows treatment locations and patterns of hospital admission based on the initial stage of presentation. Of patients with type 1 angioedema, 69.4% were discharged from the emergency room after initial treatment (and 10.7% and 5.6% for types 2 and 3, respectively). Patients with either type 2 or type 3 angioedema showed a high rate of admittance to the ward or ICU for airway monitoring. Patients with type 3 angioedema were the most often admitted to the ICU (50%, in contrast to 32.1% for type 2 and 8.0% for type 1).

TABLE 1. COMPARISON OF DEMOGRAPHICS, PRESENTATION TYPE, TIME TO IMPROVEMENT, AND TREATMENT LOCATION BETWEEN ACEI-INDUCED AND OTHER TYPES OF ANGIOEDEMA

		ACEI-Induced		*Other Causes*		
		No.	*%*	*No.*	*%*	*p*
Patients		74	68.5	34	31.5	
Race						NS
	African-American	71	95.9	27	79.4	
	White	3	4.1	4	11.8	
	Others	0	0.0	3	8.8	
Sex						NS
	Women	55	74.3	26	76.5	
	Men	19	25.7	8	23.5	
Presentation*						NS
	Type 1	43	58.1	19	55.9	
	Type 2	21	28.4	7	20.6	
	Type 3	10	13.5	8	23.5	
Time to improvement (h)						NS
	<4, Discharged from emergency room	29	39.2	18	52.9	
	<24	34	45.9	14	41.1	
	24-48	8	10.8	0	0	
	>48	3	4.1	2	5.9	
Place of treatment						NS
	Airway intervention	11	14.9	3	8.8	
	Discharged from emergency room	29	39.1	18	52.9	
	Ward	18	24.3	6	17.6	
	Intensive care unit	16	21.6	7	20.6	

ACEI — angiotensin-converting enzyme inhibitor; NS — not significant (statistical analysis performed by χ^2 test and Fisher's test).

*Type 1 — angioedema limited to face and oral cavity, excluding floor of mouth; type 2 — extension to floor of mouth and/or oropharynx (base of tongue, soft palate, uvula); type 3 — extension to supraglottic and glottic structures (epiglottis, hypopharynx, larynx).

Fourteen patients (13%) required airway intervention, all at the time of initial presentation. Twelve patients were intubated, and 2 required cricothyrotomies after initial attempts at intubation failed. Both cricothyrotomy patients presented with type 2 massive tongue and oropharyngeal swelling. Six patients each with type 2 and 3 presentations were intubated successfully. Table 3 compares the 14 patients requiring airway intervention with the 94 patients not requiring airway intervention. All were African-American (p not significant), and 71.4% were female (p not significant) in the intervention group. There was a 50% prevalence rate for diabetes ($p < .02$) and a 28.6% (p not significant) rate for asthma in the intervention group. Eleven cases (78.6%; p not significant) were attributed to ACEI use. Of those requiring intervention, 57.1% had type 2 angioedema and 42.9% had type 3 ($p < .0001$) at the initial presentation. Twenty percent of those patients who presented within 4 hours of their initial symptoms required intervention. This percentage reflected a trend ($p < .08$) in which the shorter the time elapsed before presentation, the greater the need for airway intervention.

Table 4 shows the time to improvement once treatment was initiated. There were no cases of disease progression once treatment was started. Management uniformly consisted of stopping the inciting agent, high-humidity face tents, an initial dose of antihistamines, and a continued course of intravenous steroids until the edema resolved. The majority of patients had significant improvement of their angio-

TABLE 2. PATTERN OF ADMISSION BASED ON TYPE OF ANGIOEDEMA AT INITIAL PRESENTATION

Angioedema Type	*No. of Patients*		*Discharged From ER (n = 47)*		*Admitted to Ward (n = 24)*		*Admitted to ICU (n = 18)*		*Intubated (n = 12)*		*Surgical Airway (n = 2)*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
1	62	57.4	43	69.4	14	22.6	5	8.0	0	0.0	0	0.0
2	28	25.9	3	10.7	8	28.6	9	32.1	6	21.4	2	7.1
3	18	16.7	1	5.6	2	11.1	9	50.0	6	33.3	0	0.0

ER — emergency room, ICU — intensive care unit.

TABLE 3. COMPARISON OF DEMOGRAPHICS, HISTORY OF PRIOR EPISODES OF ANGIOEDEMA, CO-MORBIDITIES, ACEI INDUCTION, AND TIME BEFORE PRESENTATION BETWEEN PATIENTS REQUIRING AIRWAY INTERVENTION AND THOSE NOT REQUIRING AIRWAY INTERVENTION

		Airway Intervention		*No Airway Intervention*		
		No.	*%*	*No.*	*%*	*p*
Patients		14		94		
Race	African-American	14	100	84	89.4	NS
Sex						NS
	Women	10	71.4	71	75.5	
	Men	4	28.6	23	24.5	
Co-morbidity						
	Asthma	4	28.6	13	13.7	NS
	Diabetes	7	50.0	18	19.1	$p < .02$
	CAD/CHF	2	14.3	19	20.2	NS
	Seizure disorder	2	14.3	3	3.2	NS
	Heart transplant	0	0.0	2	2.1	NS
ACEI-induced		11	78.6	63	67.0	NS
History of prior episode		2	14.3	29	30.9	NS
Time from onset of symptoms to presentation (h)†	<4	9/51	17.6	42/51	82.3	$p < .08$*
	4-8	3/25	12.0	22/25	88.0	
	8-12	2/13	15.4	11/13	84.6	
	>12	0/19	0.0	19/19	100	
Stage at initial presentation§	Type 1	0/62	0.0	62/62	100	NS
	Type 2	8/28	28.6	20/28	71.4	$p < .0001$
	Type 3	6/18	33.3	12/18	66.7	$p < .0001$

Statistical analysis performed by Fisher's exact test. (Asterisk indicates analysis by χ^2 test for trends.)
CAD/CHF — coronary artery disease and/or congestive heart failure.
†Percentages are of total patients presenting in that time.
§Percentages are of patients with that type of angioedema.

edema within 24 hours. Those who took from 24 to 48 hours initially presented with oropharyngeal and/or supraglottic swelling. Five patients (4.6%, $p < .0001$) with type 2 presentation, most notably base of tongue edema, took longer than 48 hours to respond. Each of these patients had an eventual resolution of his or her angioedema, and there were no reported deaths.

DISCUSSION

Originally described by Quincke in 1882, angioedema has long been recognized a serious threat to airway stability if left untreated. Angioedema may be hereditary or acquired, and much interest has surrounded the etiology of hereditary angioedema. It is an autosomal dominant condition in which the production (in 68% of cases) or function (in 32% of cases) of C1 esterase inhibitor is reduced.[6] C1 esterase inhibitor normally regulates the complement cascade, and without it, vasoactive components such as C3a and C5a are elevated. Activation of these early complement factors, along with the activation of plasminogen to plasmin, produces a unique C2 derivative called C2 kinin. C2 kinin is thought to be the cause of increased capillary permeability in hereditary angioedema.[2]

TABLE 4. HOURS UNTIL NOTICEABLE IMPROVEMENT OF ANGIOEDEMA

Angioedema Type	*<4 Hours (Discharged From ER)* *No.*	*%*	*<24 Hours* *No.*	*%*	*24-48 Hours* *No.*	*%*	*>48 Hours* *No.*	*%*
1	43	69.4	19	30.6	0	0.0	0	0.0
2	2	7.1	19	67.9	2	7.1	5	17.9*
3	2	11.1	10	55.6	6	33.3	0	0.0

Percentages are of patients with that type of angioedema.
*$p < .0001$ by Fisher's exact test.

Hereditary angioedema is rare, and in this study, there were no cases identified by either clinical history or serologic testing of complement and C1 esterase inhibitor levels. A larger series of 146 patients identified just 1 patient with hereditary angioedema.[7] Another series of 36 patients failed to identify a case of hereditary angioedema despite ordering of complete C1 esterase panels on each patient.[3] With the finding of hereditary angioedema so rare, the overwhelming majority of causes of angioedema can be considered to be acquired. Precipitating factors are diverse and include trauma, food sensitivity, food additives, temperature, and other medications such as aspirin, penicillin, and propanolol. Prior studies show ACEIs as the precipitating factor in 25% to 38% of cases.[2-5] Of our cases, 68.5% were identified as ACEI-induced. This is not a surprising number, since ACEIs have now become one of the 10 most prescribed drugs in the United States, with uses in hypertension, congestive heart failure, and diabetic renal insufficiency.[4]

Angioedema is estimated to occur in 0.1% to 0.7% of patients taking ACEIs.[1,8] In this study, the length of time a patient was taking the ACEI before an episode of angioedema was highly variable. Six patients had episodes after their first dose of ACEI, while 2 patients took ACEIs for a period of 5 years before experiencing angioedema. The average time was 14 months, with a median of 6 months. These findings corroborate other studies that showed a lack of a temporal trend in incidence beyond the first week of therapy.[1]

Although the frequency of ACEI-induced angioedema is rising, the mechanism is not yet fully understood. The effect is unpredictable, and why some patients become symptomatic while others do not remains a mystery. A high percentage of our study population is African-American. This large African-American community, with a high prevalence of diabetes and hypertension, has a high rate of usage of ACEIs. There are data suggesting that African-Americans may be at increased risk for ACEI-induced angioedema due to an increased sensitivity to bradykinin.[8]

Bradykinin levels, as well as the renin-angiotensin pathway, are influenced by ACEIs. The mechanism of ACEI-induced angioedema is now thought to be a biochemical reaction involving the renin-angiotensin pathway. The ACEIs reduce the function of angiotensin-converting enzyme (ACE), thereby altering two of its most important functions: the inactivation of bradykinin and the conversion of angiotensin I to angiotensin II.[6] Bradykinin is a nonapeptide formed from the inactive precursor kininogen by the enzyme kallikrein.[9] By reducing the function of ACE, ACEIs cause local increases in the level of bradykinin, which acts as a potent vasodilator and increases vascular permeability.[6] In addition, patients predisposed to angioedema may also have relative deficiencies of other enzymes involved with bradykinin degradation, such as kininase I (carboxypeptidase N). This is an important enzyme for bradykinin degradation, and patients who are heterozygous for this deficiency may be at greater risk of developing angioedema.[1]

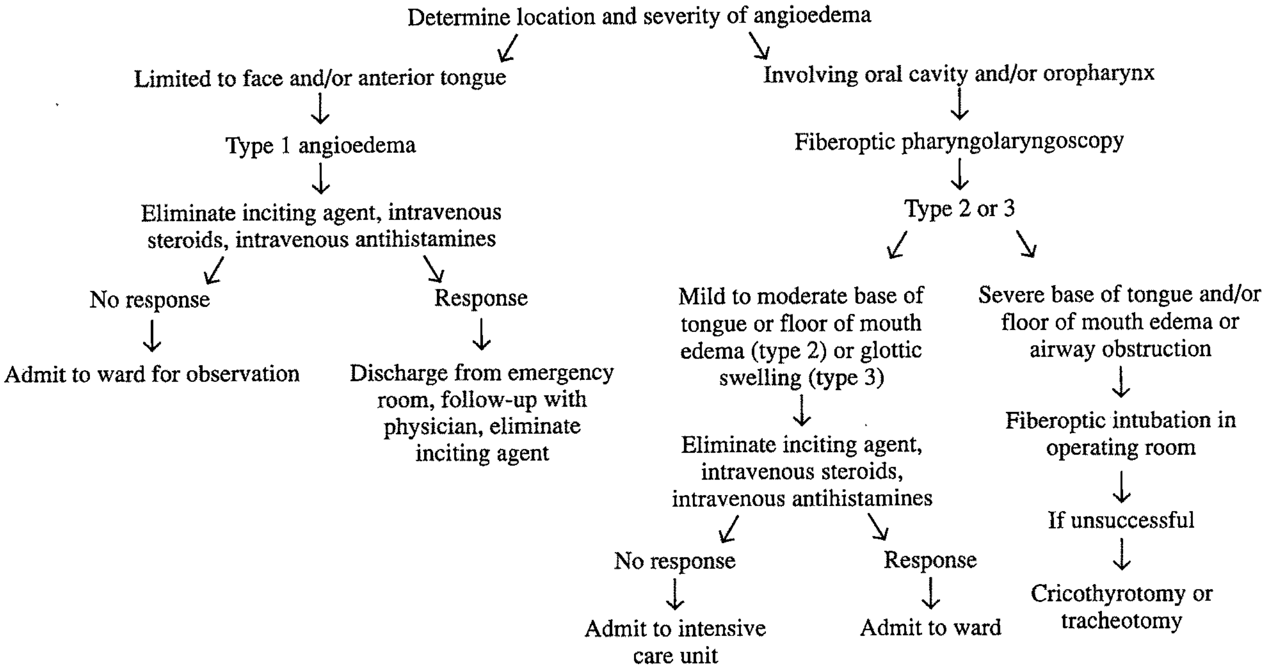
The second function of ACE is the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. Blocking the production of angiotensin II results in a net vasodilatation that produces the desired antihypertensive effect. This vasodilatation, in combination with elevated bradykinin levels, may potentiate the formation of angioedema.[6]

Because of the increase in ACEI-induced angioedema, one objective of our study was to see whether it was associated with a prognosis different from that of angioedema due to other causes. We found no significant difference in the initial presentation of patients in either group. Type 1 angioedema limited to the face and oral cavity, excluding the floor of the mouth, was the most common presentation in both groups. A slightly higher percentage of patients with ACEI-induced angioedema were admitted to the ward and ICU for monitoring, but this difference was not statistically significant. Similar percentages in each group required airway intervention. In addition, there was no significant difference between the two groups in terms of hours elapsed before noticeable improvement of the angioedema. The only significant difference noted was the preponderance of African-Americans in the ACEI group.

The most common presenting symptom of angioedema regardless of cause is facial swelling. Patients often awoke in the morning to find their lips or tongue swollen. Others present with more rapid onset of symptoms, complaining of difficulty swallowing and shortness of breath. For each angioedema patient seen, it is crucial for the entity to be recognized and for treatment to be initiated promptly. Symptoms are often clues as to the anatomic location of the edema. With type 1 angioedema, isolated facial swelling and/or anterior tongue swelling, patients quickly responded to discontinuation of the inciting agent and an intravenous dose of steroids and antihistamines. A histamine-mediated response with resultant release of bradykinin may contribute to angioedema caused by drug or food sensitivity. Although there does not appear to be a histamine-mediated cause in ACEI-induced angioedema, each patient with angioedema was initially treated with antihistamines as part of a shotgun approach regardless of cause.

In our series of 108 patients, there was no evidence of disease progression once treatment was initiated. With type 1 swelling isolated to the face and anterior tongue, patients were discharged home after a noticeable clinical response to a dose of steroids and antihistamines. Discharge instructions were given to discontinue the ACEI and consult their internists for an alternative antihypertensive medication. Oral steroids were prescribed for those with residual tongue edema.

Edema of the floor of the mouth and base of the tongue presents the most serious risk to airway stability. Twelve patients were intubated in this study,


Determine location and severity of angioedema
Limited to face and/or anterior tongue
Type 1 angioedema
Eliminate inciting agent, intravenous steroids, intravenous antihistamines
No response
Admit to ward for observation
Response
Discharge from emergency room, follow-up with physician, eliminate inciting agent
Involving oral cavity and/or oropharynx
Fiberoptic pharyngolaryngoscopy
Type 2 or 3
Mild to moderate base of tongue or floor of mouth edema (type 2) or glottic swelling (type 3)
Eliminate inciting agent, intravenous steroids, intravenous antihistamines
No response
Admit to intensive care unit
Response
Admit to ward
Severe base of tongue and/or floor of mouth edema or airway obstruction
Fiberoptic intubation in operating room
If unsuccessful
Cricothyrotomy or tracheotomy

Algorithm for management of angioedema patients based on initial presentation.

half of whom presented with type 2 angioedema involving the floor of the mouth, the base of the tongue, and the oropharynx. Two additional patients, both with massive tongue and soft palate edema, underwent emergent cricothyrotomies after initial attempts at fiberoptic intubation were unsuccessful. It is our belief that with significant base of tongue and supraglottic edema distorting anatomic landmarks, each patient requiring airway intervention should be fiberoptically intubated in a controlled setting, ideally, the operating room. If the intubation attempt is unsuccessful, a tracheotomy or cricothyrotomy should be performed. Medical therapy consisting of intravenous steroids and discontinuation of the inciting agent should be instituted immediately and continued until the edema is resolved and the patient is able to be extubated or decannulated.

The analysis of those patients requiring airway intervention revealed a significant prevalence of diabetes. This finding may imply a role of high serum glucose levels in severe angioedema, but it may also just reflect the high incidence of diabetics on ACEIs. There was a trend in which those requiring intervention presented within 4 hours of their initial symptoms. In addition, there were no patients who required intervention and presented more than 12 hours after their initial symptoms.

While the majority of patients with type 1 angioedema are discharged from the emergency room, patients with type 2 angioedema are much slower to respond to treatment. Of the 61 patients admitted to the hospital for observation, 5 took longer than 48 hours to improve. Each of these 5 patients had initially presented with major base of tongue and floor of mouth edema. Because of this slower response to treatment and the anatomic obstruction it poses, we have a lower threshold for intubation of patients with type 2 angioedema and are more apt to monitor them in an ICU setting until their edema begins to resolve.

Type 3 angioedema involves extension to supraglottic and glottic structures. Each of the 18 patients with laryngeal involvement also exhibited soft palate, uvula, or base of tongue edema. There were no cases of isolated supraglottic or laryngeal involvement. Six patients required intubation, and 9 others were monitored in the ICU. Once medical treatment is initiated, glottic and supraglottic edema are often quick to respond. Sixty-seven percent of these patients showed marked improvement in less than 24 hours, while the remaining 33% had improved by 48 hours. We recommend that if a patient presents with laryngeal and/or supraglottic edema, and has no obstruction or signs of respiratory fatigue, a trial of steroids and antihistamines should be given before intubation is attempted. If the patient does not respond to treatment within an hour, shows signs of respiratory fatigue, or has potentially obstructing base of tongue or floor of mouth edema, fiberoptic intubation should be attempted (see Figure).

CONCLUSION

Angioedema often involves harmless facial and oral cavity swelling, but if left untreated, it may lead to life-threatening airway compromise. The increasing popularity of ACEI use has brought about a higher prevalence of ACEI-induced angioedema. For proper treatment of all angioedema patients, it is crucial to recognize this entity and initiate prompt therapy based on the presenting signs and the anatomic location of the edema. Angioedema involving the base of the tongue and/or the oropharynx poses the most serious risk to airway stability. Patients with angioedema of these sites are also slower to respond to medical therapy, need to be monitored more closely, and are at a higher risk for the need for airway intervention.

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CENTRAL INCISOR WIDTH AS A PREDICTOR OF APPROPRIATE CURETTE SIZE IN ADENOIDECTOMY

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As inadvertent eustachian tube injury during adenoidectomy can have serious short- and long-term implications, a simple anatomic correlate that could predict the optimal curette choice for adenoidectomy, especially in severely hypertrophic cases, is beneficial. This study evaluates the correlation of the distance between the lateral borders of the upper central incisors and the distance between the tori tubarius in the nasopharynx. One hundred one consecutive patients undergoing adenoidectomy at a pediatric tertiary care hospital were enrolled in this study. The patients ranged in age from 7 months to 15 years. No complications were noted in any of the procedures. During the operation, the distance between the central upper incisors was measured in millimeters, as was the inter-tubarius width (ITW). A multiple regression analysis was completed to assess the correlation between central incisor width and ITW. Age and inter-incisor width were positively correlated with ITW in a statistically significant manner ($p = .007$ and $p = .006$, respectively). The distance between the lateral borders of the upper central incisors predicts the distance between the tori tubarius in the nasopharynx. Therefore, an adenoid curette the window of which does not overlap the lateral aspects of the central upper incisors can be used relatively safely, even in fields with poor visualization.

KEY WORDS — adenoidectomy, central incisor width, eustachian tube dysfunction, inter-tubarius width.

INTRODUCTION

Adenoidectomy is one of the most commonly performed operations for children in the United States. Complications associated with the procedure are uncommon, yet do occur. Sequelae such as velopharyngeal incompetence,[1] persistent hypernasal speech,[2] nasopharyngeal stenosis,[3] and atlantoaxial subluxation[4] are well documented in the literature. Various techniques also exist for performing the operation. Methods for removal of adenoid tissue via laser ablation,[5] endoscopic microdebridement,[6] and electrocautery[7] have been described.

Eustachian tube injury is an unusual complication of adenoidectomy.[4,8] Such injury may be directly related to adenoid removal, subsequent hemostatic efforts with electrocautery, and underlying factors such as reflux of gastric fluids into the nasopharynx. However, injudicious use of a curette or other instruments within the nasopharynx may result in injury to the torus tubarius, leaving the patient with permanent eustachian tube dysfunction and its associated morbidity. The choice of proper curette size during adenoidectomy is usually based upon patient size and visualization of the nasopharynx. Yet, if the adenoids are exceedingly large, an adequate view of the instrument's lateral borders and the eustachian tubes may be obscured. In such cases, inadvertent injury could occur if too large a curette is used.

We therefore sought a clinical correlate that could readily predict the proper curette size with acceptable accuracy and potentially decrease the risk for eustachian tube trauma. We hypothesized that the distance between the lateral borders of the upper central incisors might approximate the distance between the eustachian tube openings in the nasopharynx. This prospective study was undertaken to address this question.

METHODS AND MATERIALS

Between November 1998 and March 1999, 101 patients underwent adenoidectomy at an urban tertiary care pediatric hospital. The procedure was performed separately or in combination with tonsillectomy and/or myringotomy tube placement. There were 55 boys and 46 girls in the study. The age range was 7 months to 15 years. In all cases, the operation was performed with a Barnhill adenoid curette, sizes 00 through 3. Adenoidectomy was completed in the standard fashion. After all adenoid tissue had been removed, the nasopharynx was dried in the midline with suction Bovie electrocautery and copiously irrigated with saline solution to achieve the best possible visual-

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TABLE 1. DIAMETERS OF BARNHILL ADENOID CURETTES

Curette No.	*Inner (mm)*	*Outer (mm)*
00	8	10
0	12	14
1	12	15
2	15	19
3	18	21

ization of the nasopharynx. Next, the adenoid curettes were placed in the midline of the nasopharynx, between the tori tubarius of the eustachian tubes. The curette that fit in such a way that its lateral borders were parallel to each fossa of Rosenmueller (ie, did not overlap either torus tubarius) was recorded. This measurement was taken to be the inter-tubarius width (ITW). The inner and outer diameters of the curettes are seen in Table 1.

The lateral borders of the central incisors were marked on a tongue blade with a pen, and the distance between them was measured and recorded. Patient age, gender, and dentition (primary versus secondary) were also recorded. The range of central incisor width was 12 to 21 mm. The range of ITW was 10 to 21 mm. Children missing one or both central incisors were excluded from the study.

RESULTS

All patients tolerated their procedures well and had no postoperative complications. A multiple regression analysis was conducted to evaluate how well patient characteristics predicted ITW. The predictors used were age, gender, dentition, and central incisor width. Indices used to indicate the relative strength of the individual predictors are presented in Table 2. All the bivariate correlations were positive, although only 2 of the 4 indices were statistically significant ($p < .01$). Patient age and central incisor width both positively correlated with ITW in a statistically significant manner. The strength of the bivariate correlation between patient age and ITW was .61 ($p = .007$). Additionally, the bivariate correlation between central incisor width and ITW was also significant (.597, $p = .006$). On the basis of this correlational analysis, central incisor width is a useful predictor of ITW. Therefore, the largest curette that can be placed over the upper central incisors, the window of which does not overlap their lateral borders, can be placed in the midline of the nasopharynx for relatively safe adenoidectomy. The distribution of appropriate curette size related to inter-incisor width is shown in the Figure.

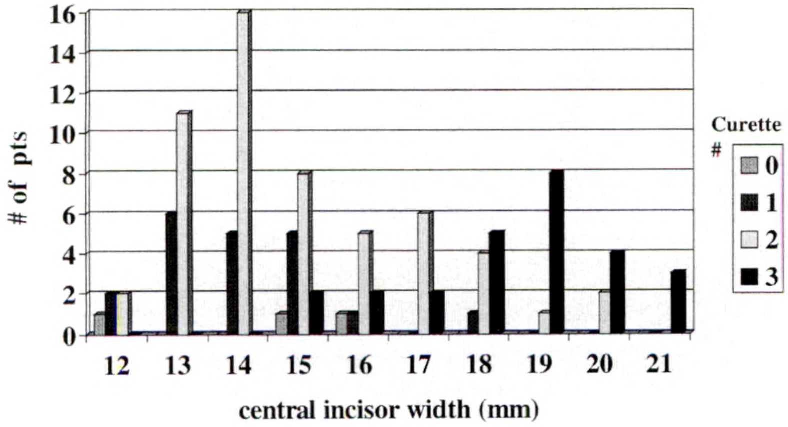
TABLE 2. CORRELATIONS BETWEEN PREDICTORS AND CRITERION VARIABLES

Predictors	*Correlations Between Predictors and ITW*	*Partial Correlations Between Predictors and ITW Controlling for All Other Predictors*
Age	.61*	.27*
Gender	.04	.11
Dentition	.58	.01
Central incisor width	.60*	.28*

ITW — inter-tubarius width.
*p < .01.

DISCUSSION

Eustachian tube injury is a rare complication of adenoidectomy. Yet, when it occurs, sequelae such as chronic otitis media with effusion, tympanic membrane retraction, and conductive hearing loss may result, secondary to permanent eustachian tube dysfunction.[4] Factors such as poor visualization, multiple passes in a bloody field, imprudent suction cautery, and reflux disease can increase the risk to structures adjoining the adenoid bed. Even with direct visualization of the nasopharynx during curettage adenoidectomy, hypertrophic adenoid tissue can obscure the surgeon's view of the tori tubarius and fossa of Rosenmueller, rendering proper curette selection difficult. It is for a situation such as this that we sought



Distribution of appropriate curette size as related to inter-incisor width for 101 study patients.

a clinical correlate that could predict proper curette size.

The finding that age is a predictor of ITW is not surprising. It is this correlation that many surgeons may use when choosing a curette to perform adenoidectomy. The older and larger the child, the larger the curette. However, age as a predictor of ITW does not provide the surgeon with an actual measurement, as does the central incisor width. By placing several instruments over the central incisors and selecting the largest curette the window of which does not overlap the lateral borders of the central incisors, the surgeon can predict with reliability that the distance between the eustachian tubes will not be exceeded. This simple maneuver decreases the potential for injury to the tori tubarius.

CONCLUSION

Central incisor width is a useful predictor of ITW. The measurement is simple, reliable, and easily obtainable within the operative field. This anatomic correlation can be used to increase safety, and to prevent injury to the eustachian tube entrance during adenoidectomy.

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BACTERIOLOGY OF ADENOIDS AND TONSILS IN CHILDREN WITH RECURRENT ADENOTONSILLITIS

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Adenoids and tonsils electively removed from 25 children with a history of recurrent group A β-hemolytic streptococci (GABHS) adenotonsillitis were cultured for aerobic and anaerobic bacteria. Two hundred twenty-four organisms (112 aerobes and facultatives, 110 anaerobes, and 2 *Candida albicans*) were isolated from the tonsils, and 229 (111 aerobes and facultatives and 118 anaerobes) were isolated from the adenoids. Mixed infection was present in all instances, with an average of 9.1 isolates per specimen. The predominant aerobes were *Streptococcus* sp, *Haemophilus influenzae,* and GABHS, and the prevalent anaerobes were *Peptostreptococcus, Prevotella,* and *Fusobacterium* spp. β-Lactamase–producing bacteria were detected in 72 isolates recovered from 22 tonsils (88%) and in 74 isolates recovered from 21 adenoids (84%). Discrepancies in the organisms recovered were found between the tonsils and adenoids. Of the aerobic isolates, 20% were isolated only in tonsils and 18% only in adenoids. Of the anaerobes, 20% were found only in tonsils and 26% only in adenoids. This study demonstrates a polymicrobial aerobic-anaerobic flora in both adenoids and tonsils, and discrepancies in recovery of pathogens such as GABHS. The adenoids may serve as a potential source of tonsillitis caused by GABHS.

KEY WORDS — adenoids, anaerobic bacteria, β-lactamase, *Haemophilus influenzae, Streptococcus* group A, tonsil.

INTRODUCTION

Adenoidectomy and tonsillectomy are frequently performed to prevent recurrent group A β-hemolytic streptococci (GABHS) adenotonsillitis.[1] Patients are usually treated with multiple courses of penicillin; however, many continue to harbor GABHS in their pharynx.[2]

One explanation for the failure of penicillin in eradicating the infection is that its repeated administration results in a shift in the tonsillar microflora, with the selection of β-lactamase–producing bacteria (BLPB).[2-4] It is postulated that by degrading penicillin at the site of infection, BLPB may protect not only themselves, but also GABHS.[2]

The core tonsillar aerobic and anaerobic flora of patients with recurrent tonsillitis has more BLPB[5] and higher concentrations of microorganisms than are found in uninflamed tonsils.[6] However, the aerobic and anaerobic flora of concomitantly removed adenoids has not been studied before, and therefore, no correlation has been established between the organisms isolated in the tonsils and those recovered from the adenoids.

The purpose of the present study was to determine and compare the bacterial flora of recurrently inflamed adenoids and tonsils in children with adenotonsillitis.

PATIENTS AND METHODS

The adenoids and tonsils were removed from consecutively seen pediatric patients admitted for elective adenoidectomy and tonsillectomy. Twenty-five children were studied (1 adenoid and 1 tonsil from each child). Their ages ranged from 4 to 15 years (mean, 7 years 6 months), and 14 were boys. They suffered from recurrent adenotonsillitis, defined as at least 5 episodes of inflammation of the tonsils within 1 year during which GABHS were recovered. The duration of the patients' symptoms ranged from 10 months to 3 years (average, 15 months). They had received multiple courses of antibiotics, but none had received antimicrobial therapy for at least 1 month before surgery. Immediately after excision, the adenoids and tonsils were placed in a sterile container and taken to the anaerobic microbiology laboratory. The right adenoid and tonsil were placed in a sterile petri dish and held by forceps. One side was cauterized with a heated scalpel, and an incision was made through that area, cutting the adenoid or the tonsil in half. The cores were swabbed with a sterile cotton-tipped applicator and plated onto aerobic and anaerobic media. The time between removal of the adenoids and tonsils and inoculation of the specimens was never in excess of 15 minutes.

Specimens were inoculated onto 5% sheep blood, chocolate, and MacConkey's agar plates for aerobic and facultative organisms. The plates were incubated at 37°C aerobically (MacConkey's) or under 5% carbon dioxide (5% sheep blood and chocolate) and examined at 24 and 48 hours. For anaerobes, the material was plated onto prereduced vitamin K_1–enriched

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TABLE 1. AEROBIC AND FACULTATIVE ORGANISMS ISOLATED FROM CORE OF EXCISED TONSILS AND ADENOIDS FROM 25 CHILDREN

	No. of Isolates (No. of BLPB) in Tonsils and Adenoids				
Organism	*Tonsils Total*	*Adenoids Total*	*Tonsils Only*	*Adenoids Only*	*Tonsils and Adenoids*
Gram-positive cocci					
Streptococcus pneumoniae	3	2	1	0	2
α-Hemolytic *Streptococcus*	22	18	6	2	16
γ-Hemolytic *Streptococcus*	16	17	2	3	14
β-Hemolytic *Streptococcus*					
Group A	8	9	4	5	4
Group B	1	2		1	1
Group C	2	3		1	2
Group F	3	2	2	1	1
Staphylococcus aureus	13 (13)	12 (12)	3 (3)	2 (2)	10 (10)
Staphylococcus epidermidis	3 (3)	1 (1)	2 (2)		1 (1)
Gram-negative cocci					
Moraxella catarrhalis	10 (10)	12 (12)	1 (1)	3 (3)	9 (9)
Gram-positive bacilli					
Lactobacillus spp	3	4	0	1	3
Diphtheroid spp	4	3	1		3
Gram-negative bacilli					
Haemophilus influenzae					
Type b	2 (1)	3 (2)	0	1 (1)	2 (1)
Non–type b	15 (11)	17 (13)	0	2 (2)	15 (11)
Haemophilus parainfluenzae	3 (2)	4 (2)	1 (1)	2 (1)	2 (1)
Eikenella corrodens	2	1	1	0	1
Pseudomonas aeruginosa	1 (1)	0	1 (1)	0	0
Escherichia coli	1	1	1	1	0
Yeasts					
Candida albicans	2	0	2	0	0
Total	114 (41)	111 (42)	28 (8)	25 (9)	86 (33)

*BLPB — β-lactamase–producing bacteria.

Brucella blood agar, onto an anaerobic blood agar plate containing kanamycin sulfate and vancomycin hydrochloride, onto an anaerobic blood plate containing colistin and nalidixic acid, and into an enriched thioglycollate broth (containing hemin and vitamin K1).[7,8] The anaerobic plates and thioglycollate broth were incubated in anaerobic jars and examined at 48 and 96 hours. Organisms were identified by techniques previously described.[7,8]

β-Lactamase activity was determined on all organisms by the chromogenic cephalosporin analog 87/132 method.[9] Statistical analysis comparing the groups was done with a randomization test.[10]

RESULTS

Two hundred twenty-four organisms (112 aerobes and facultatives, 110 anaerobes, and 2 *Candida albicans*) were isolated from the tonsils (Tables 1 and 2). Mixed aerobic and anaerobic flora was present in all the tonsils, yielding an average of 9.0 isolates (4.5 aerobes, 4.4 anaerobes, and .08 *C albicans*) per specimen.

Two hundred twenty-nine organisms (111 aerobes and facultatives and 118 anaerobes) were recovered from the adenoids. A mixed flora was present in all adenoids, and the average number of isolates was 9.2 (4.4 aerobes and 4.7 anaerobes) per specimen. No consistent pattern of combinations of the different aerobic and anaerobic bacteria was noted at either site.

The aerobic organisms most frequently isolated in both sites were α-hemolytic streptococci, *Staphylococcus aureus*, β-hemolytic streptococci (groups A, C, and F), *Moraxella catarrhalis*, *Haemophilus* spp, and γ-hemolytic streptococci. The predominant anaerobic organisms were *Prevotella* spp, *Bacteroides fragilis*, *Fusobacterium* spp, anaerobic gram-positive cocci, and *Veillonella parvula*.

The production of BLPB was detectable in 72 isolates recovered from 22 tonsils (88%) and in 74 isolates recovered from 21 adenoids (84%; Tables 1 and 2). These included all isolates of *Staphylococcus* spp, *M catarrhalis*, and *B fragilis* group, 27 of 37 isolates (73%) of *H influenzae*, 4 of 7 (57%) of *Haemophilus*

TABLE 2. ANAEROBIC BACTERIA ISOLATED FROM CORE OF EXCISED TONSILS AND ADENOIDS FROM 25 CHILDREN

	No. of Isolates (No. of BLPB) in Tonsils and Adenoids				
Organism	*Tonsils Total*	*Adenoids Total*	*Tonsils Only*	*Adenoids Only*	*Tonsils and Adenoids*
Gram-positive cocci					
Peptostreptococcus spp	3	3	0	0	3
Peptostreptococcus asaccharolyticus	2	3	1	2	1
Peptostreptococcus magnus	3	3	1	1	2
Peptostreptococcus anaerobius	2	2	1	1	1
Peptostreptococcus intermedius	2	3	0	1	2
Peptostreptococcus micros	2	0	2	0	0
Peptostreptococcus constellatus	2	3	1	2	1
Microaerophilic streptococci	5	7	0	2	5
Gram-negative cocci					
Veillonella parvula	11	13	2	4	9
Gram-positive bacilli					
Bifidobacterium adolescens	2	1	1	0	1
Eubacterium spp	5	4	2	1	3
Lactobacillus spp	1	1	1	1	0
Propionibacterium acnes	0	1	0	1	0
Actinomyces spp	2	2	1	1	1
Gram-negative bacilli					
Fusobacterium spp	3 (1)	4 (2)	1	2 (1)	2 (1)
Fusobacterium mortiferum	1	0	1	0	0
Fusobacterium necrophorum	2 (1)	2 (1)	0	0	2 (1)
Fusobacterium nucleatum	16 (8)	18 (9)	3 (1)	5 (2)	13 (7)
Bacteroides spp	6 (2)	6 (2)	2 (1)	2 (1)	4 (1)
Porphyromonas asaccharolytica	3 (1)	4 (2)	0	1 (1)	3 (1)
Prevotella melaninogenica	18 (7)	17 (6)	5 (2)	4 (1)	13 (5)
Prevotella intermedia	6 (2)	8 (3)	0	2 (1)	6 (2)
Prevotella oralis	3 (1)	4 (1)	1	2	2 (1)
Prevotella oris	2	3	0	1	2
*Bacteroides fragilis**	2 (2)	3 (3)	1 (1)	2 (2)	1 (1)
*Bacteroides distasonis**	2 (2)	1 (1)	1 (1)	0	1 (1)
*Bacteroides vulgatus**	1 (1)	0	1 (1)	0	0
*Bacteroides uniformis**	1 (1)	1 (1)	0	0	1 (1)
*Bacteroides ureolyticus**	2 (2)	1 (1)	1 (1)	0	1 (1)
Total	110 (31)	118 (32)	30 (8)	38 (9)	80 (23)

**Bacteroides fragilis* group.

parainfluenzae, 22 of 46 (48%) of *Fusobacterium* spp, 20 of 61 (33%) of *Prevotella* spp, and 3 of 7 (43%) of *Porphyromonas asaccharolytica*.

Comparison between the tonsillar and adenoidal specimens showed similarities and discrepancies between them (Tables 1 and 2). Of the total 139 aerobic bacterial isolates recovered from the adenoids and tonsils, 28 (20%) were recovered only in tonsils, 25 (18%) only in adenoids, and 86 (62%) in both locations. Of the total 148 anaerobic isolates recovered from these locations, 30 (20%) were found only in tonsils, 38 (26%) only in adenoids, and 80 (54%) in both locations.

There was generally no statistically significant difference in the recovery rate of organisms between the sites. However, in the case of β-hemolytic streptococci, concordance between adenoids and tonsils was only present in 8 patients; 8 isolates were found only in adenoids and 6 isolates only in tonsils (Table 1).

DISCUSSION

This study confirms the polymicrobial aerobic and anaerobic flora of the core of recurrently inflamed tonsils[11-14] and adenoids.[15] As was previously noted,[16] we also found a high prevalence of recovery of *H influenzae* in the adenoids and tonsils. This study also highlights the importance of BLPB, which were detected in 84% of the adenoids and 88% of the tonsils. This finding may be attributable to the effects of previous penicillin therapy, which may select for or induce the emergence of these organisms.[3,4,17]

The existence of aerobic and anaerobic BLPB within the cores of the adenoids and tonsils may explain the persistence of many pathogenic organisms in the cores, in which the organisms may be shielded from the activity of penicillin.[18] The disappearance of these bacteria between episodes of tonsillitis may be only temporary, and their reappearance may be due to their reemergence from the cores of the adenoids or tonsils. The discrepancies in culture results between the cores of adenoids and the cores of tonsils in the recovery of GABHS gives credence to this possibility. If the GABHS survive within the adenoids, they can reinfect the tonsils, causing a seemingly new episode of tonsillitis. Because cultures of the adenoids are not taken routinely, the persistence of GABHS in the adenoids after completion of penicillin therapy may not be detectable. Our findings suggest that obtaining adenoidal cultures from patients with recurrent tonsillitis may reveal a potential source of this organism. Finding GABHS may prompt attempts to eliminate them in those patients who harbor them.

This study demonstrates for the first time the overall similarity in the aerobic and anaerobic bacterial flora of the adenoids and tonsils. A potential synergy between these organisms is possible. In vivo synergy has been demonstrated between GABHS and several aerobic and anaerobic bacteria, including *H influenzae*, *S aureus*, pigmented *Prevotella* spp, *Bacteroides* spp, *Fusobacterium* spp, and *Peptostreptococcus* spp.[19] Whether such synergy is operative in inflamed adenoids and tonsils has yet to be determined.

Our findings underscore the importance of continuous monitoring of bacterial isolates from recurrently inflamed adenoids and tonsils and their antimicrobial resistance patterns. The antimicrobials that exhibit efficacy superior to that of penicillin in eradicating GABHS — as well as both aerobic and anaerobic BLPB — are clindamycin hydrochloride[20,21] and the combination of amoxicillin and clavulanic acid.[22,23] Further studies are warranted to evaluate the effect of these and other agents on patients who harbor GABHS in their adenoids.

ACKNOWLEDGMENTS — The authors thank Drs E. M. Friedman and J. J. Kuhn for providing adenoids and tonsils, L. Calhoun, J. E. Perry, and J. C. Gilmore for technical support, and Justine Rosario for secretarial assistance.

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MANAGEMENT OF THE TINNITUS PATIENT

The Ninth Annual Conference on the Management of the Tinnitus Patient, for professionals and tinnitus patients, will be held in Iowa City, Iowa, October 4-6, 2001. For information, see web site www.medicine.uiowa.edu/otolaryngology/news/news or contact Rich Tyler by telephone (319-356-2471) or e-mail (rich-tyler@uiowa.edu).

Ann Otol Rhinol Laryngol 110:2001

PREFABRICATION OF COMPOSITE TISSUE FOR IMPROVED TRACHEAL RECONSTRUCTION

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Tracheal repair tissues were evaluated experimentally to provide an evidence-based choice for decision-making in clinical tracheal reconstruction. Tracheal reconstructive tissue was characterized as providing for vascularization, support, and/or lining. A tissue equivalent was developed in the rabbit for each of the individual tissues. The individual tissues consisted of nonepithelialized soft tissue (vascularized fascia), epithelialized tissue (vascularized fascia grafted with buccal mucosa), and supportive tissue (ear cartilage). The 3 reconstructive tissues were evaluated in 30 rabbits after repair of an anterior laryngotracheal defect. Morphometric and histologic analysis was applied to the reconstructions. After a 1-month follow-up period, defects repaired with nonepithelialized soft tissue showed healing by secondary intention and a wound that was contracted to 44% of the initial surface area of the defect. Mucosa-lined soft tissue flaps and cartilage grafts showed a less than 10% wound contraction. Compared to cartilage grafts, mucosa-lined soft tissue (vascularized fascia grafted with buccal mucosa) seemed preferable for clinical use, because it showed healing by primary intention. A mucosa-lined radial forearm fascia flap was used successfully in cases of restenosis after tracheal resection. One deficiency of the mucosa-lined soft tissue was the absence of supportive tissue. In cases of extensive stenosis, it might be useful to obtain additional expansion of the airway lumen by creating a convexity at the site of reconstruction. In a second set of experiments, we attempted to improve the mucosa-lined soft tissue concept by adding elastic cartilage. A composite tissue consisting of vascularized fascia, buccal mucosa, and auricular cartilage was developed. Heterotopic prefabrication of the composite tissue was essential for survival of the cartilaginous component. Additional airway lumen expansion could be obtained after heterotopic flap prefabrication. After experimental evaluation, the concept of the prefabricated composite tissue was successfully applied in a clinical case of long-segment stenosis. Experimental and clinical evidence suggests that the combination of buccal mucosa and fascia form an optimized tissue combination for tracheal reconstruction. This combination can be improved by adding strips of autologous ear cartilage.

KEY WORDS — augmentation, composite tissue, prefabricated flap, stenosis, tissue, trachea.

INTRODUCTION

The best external surgical approach for tracheal stenosis is segmental resection with end-to-end anastomosis.[1] Augmentation of the tracheal lumen by inserting local, regional, or distant tissue is necessary when tracheal resection is not possible, as, for example, in long-segment stenoses (more than half of the tracheal length) or in cases of restenosis after tracheal resection. A stenosis of more than half the tracheal length is rather exceptional. However, because the indications for tracheal resection are growing, it can be anticipated that restenosis at the anastomotic site will become a more frequently encountered problem.[2-4] Restenosis poses a therapeutic problem because further resection is usually not possible and, usually, reconstructive tissue has to be used to augment the insufficient airway segment.

Tracheal reconstruction with repair tissue is a second-choice solution, because the optimal repair tissue is not available clinically. Optimal tracheal repair tissue should resemble the native tracheal tissue as closely as possible and be composed of a cartilaginous support, an internal lining consisting of respiratory mucosa, and a reliable blood supply. This optimal repair tissue cannot be found outside the airway. As a consequence, all the currently used reconstructive options are missing one or more basic requirements.

The most frequently used reconstructive tissues for tracheal lumen augmentation consist of cartilage grafts[5] and muscle flaps that are used as a carrier for skin,[6] periosteum,[7] or bone.[8] Results obtained with these reconstructive tissues are not consistent, because they all lack one or more of the requirements for optimal tracheal repair. Moreover, the choice between the different reconstructive options is difficult, because no valuable comparison is available. Because the disorder is so rare, all the reconstructive possibilities have been applied in series with small numbers of patients, without clear definition of the repaired defects, and with the number of decannulated cases as the only outcome variable.

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A possibility for making an evidence-based choice between the different reconstructive options may lie in a well-controlled experimental comparison model. We therefore characterized the reconstructions by their tissue components, consisting of vascularization, support, and lining. The basic tissue requirements were translated into specific rabbit tissues that could be used for experimental comparison after repair of airway defects.

It is important in tracheal reconstruction to obtain primary wound healing with a minimal amount of wound contraction and to obtain an airway lumen sufficient for normal respiration. We attempted to compare the different individual and composite repair tissues in their capacity to repair an anterior airway defect. From the experimental results obtained with this model, an optimized reconstructive concept was developed and successfully used in clinical cases of tracheal stenosis.

EXPERIMENTAL EVALUATION OF INDIVIDUAL REPAIR TISSUES

MATERIAL AND METHODS

The capacity of the different reconstructive tissues in preserving the surface area of an anterior laryngotracheal airway defect was evaluated. We used rabbit ear cartilage as supportive tissue and oral mucosa as internal lining. A vascularized fascia flap (lateral thoracic fascia) was used as nonepithelialized soft tissue and as a vascular carrrier for the mucosal graft (Fig 1). The vascularized fascia flap was preferred as vascularized tissue because it provides for the thinnest axially perfused flap that is currently available.

Repair Tissues. In group 1 (10 animals), fascia, which provides vascularization, was used. An area of 1.5 × 0.8 cm of the rabbit's lateral thoracic fascia[9] was used to close the airway defect (Fig 1). The lateral thoracic fascia flap is located in the lateral thoracic area and is axially perfused by the lateral thoracic artery and vein. The flap is transferable to the neck region by subcutaneous transposition on the vascular pedicle.

In group 2 (10 animals), cartilage was used to provide support. A cartilage plate (1.5 × 0.8 cm) with preserved perichondrium at each side was taken at the root of the outer ear.

In group 3 (10 animals), fascia and buccal mucosa were used to provide vascularization and lining tissue. Two full-thickness mucosal grafts (0.75 cm × 0.8 cm) were taken at the left and right buccal areas. The lateral thoracic fascia flap was dissected, and the 2 mucosal grafts were sutured to the distal end of the fascial flap to obtain a mucosa-covered area of 1.5 × 0.8 cm (Fig 1). The mucosa-lined fascia was isolated on its vascular pedicle, rotated to the neck, and sutured into the anterior airway defect.

Airway Defect. The animals were premedicated, and anesthesia was maintained with halothane through mask ventilation. The cervical trachea was reached through a midline neck incision, and the upper end was dissected over 2 cm from the surrounding tissues. An anterior laryngotracheal defect (measuring 1.5 × 0.8 cm) was created at the level of the cricoid cartilage and at the first tracheal rings (Fig 1). A photograph was taken of the anterior defect. For the different reconstructions, a patch of a size similar to that of the defect was sutured into the defect with 5-0 polypropylene (Prolene). The skin incisions were closed, and the rabbits were allowed to breathe without stent or tracheotomy. They were followed for 4 weeks.

Thirty mature New Zealand White rabbits, with a mean age of 6 months and weighing between 3 and 3.5 kg, were used. Rabbits were housed in separate quarters in an environment-controlled facility and fed a standard rabbit diet. All animals received humane care in compliance with the *Principles of Laboratory Animal Care* formulated by the US National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the US National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Evaluation. After the follow-up period, the animals were killed painlessly with an overdose of sodium pentobarbital, and the laryngotracheal complex was excised. Animals in which a fascia flap was used (groups 1 and 3) underwent silicone dye injection of the lateral thoracic artery before tracheal harvest to permit better visualization of the revascularization process. Therefore, the lateral thoracic region was opened, and the left thoracic artery of the fascia flap was flushed through the cannula with 10 mL of heparinized normal saline solution (10 IU of heparin per milliliter) followed by 5 mL of blue silicone dye (Microfil, Canton Bio-Medical Products, Inc, Boulder, Colo). This material formed a cast of the vascular architecture of the fascia flap and the established vascular connections with the tracheal patch.

All 30 harvested tracheas were incised longitudinally at their posterior side, pinned onto cardboard, and photographed with a ruler in place. The photographs of the inner side of the reconstruction and the photographs of the defects (taken during the initial operation) were scanned on a color scanner and digitized into a computer. These color pictures were displayed on a screen for tracing and calculation. The surface area of the defect and the surface area existing after reconstruction were outlined with a computer mouse. From these measurements, the percentage reduction in surface area (SAR) could be calculated: $SAR = SA_f - SA_f/SA_i$, where SA_f and SA_i are the final and initial surface areas of the reconstruction, respectively.

For the overall comparison of the percentages of the reconstructed area for the 3 reconstructions, a Kruskal-Wallis test was used. Statistical significance for the test was set at $p < .05$.

The different reconstructions were evaluated macroscopically and histologically (axial sections). The tissues were embedded in paraffin, and 4-µm-thick axial sections were cut and stained with hematoxylin-eosin.

RESULTS

In order to have a group of 30 animals to follow for a 4-week period, we had to operate on 35 animals. Five animals died during follow-up for reasons not related to their reconstruction. All the reconstructed areas of group 1 and group 3 animals were vascularized by the fascia flap and showed a blue coloration after Microfil injection (Fig 2A,C).

The surface area of the reconstruction was well preserved for group 2 and group 3 animals. The mean size reduction of the reconstructed surface area was 5.2% (SE, 3.1%) in group 2 animals and 8.1% (SE, 2.9%) after reconstruction with mucosa-lined fascia. No statistical difference in wound contraction was found between group 2 and group 3 animals. In group 1, the mean size reduction of the reconstructed surface area measured 44.5% (SE, 10.2%) and was significantly higher than in groups 2 and 3. Histologically, the nonepithelialized reconstructions (groups 1 and 2) proved to be lined with respiratory epithelium by the time of evaluation. In group 1, remucosalization started at the margins of the graft and involved progressive migration of the epithelium to the center of the graft over the 1-month follow-up period. After the follow-up period, the contracted surface area in group 1 was completely covered with respiratory epithelium.

The cartilage grafts (group 2) underwent revascularization and re-epithelialization from the margins of the defect. Regenerated submucosal blood vessels and respiratory epithelium were less developed in the middle of the graft (Figs 2B and 3). The mucosa-lined reconstructions (group 3) showed primary healing with complete take of the oral mucosa on the fascial vascular carrier (Figs 2C and 4).

DISCUSSION

Soft tissue without epithelial lining will heal by secondary intention when used inside the airway. This tissue undergoes re-epithelialization in combination with a wound contraction process and with a significant reduction of the reconstructed surface area. Examples of clinically used tissues within this group are periosteum[7] and muscle.[10] Because of the wound contraction process, soft tissues without epithelial lining are not a first-choice option when augmentation of a stenotic airway is attempted.

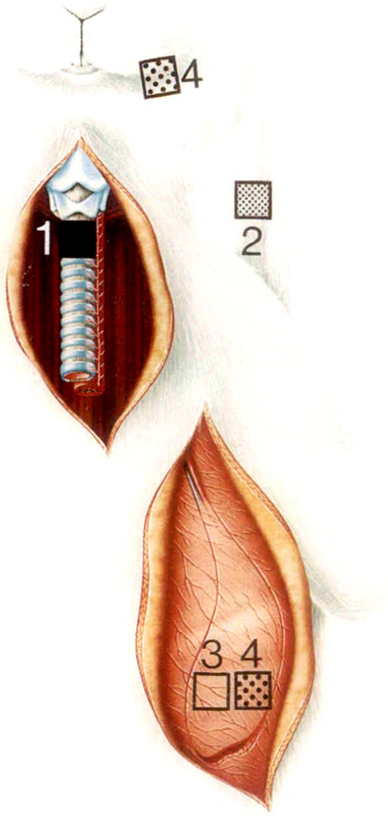

Fig 1. Reconstruction of anterior laryngotracheal defects. Defect of 1.5 cm (length) by 0.8 cm (width) is created at level of cricoid cartilage and upper trachea (1). This defect is repaired with piece (0.8 × 1.5 cm) of auricular cartilage (2) or with segment of lateral thoracic fascia flap (3). Fascia flap can be used as nonepithelialized tissue or as epithelial-lined fascia after application of full-thickness buccal mucosa graft (4). Area of 1.5 × 0.8 cm, with or without full-thickness mucosa, is sutured into anterior laryngotracheal defect.

Cartilage patches undergo secondary healing with re-epithelialization and revascularization induced from the margins of the defect. Both clinical and animal studies have established that autologous costal and auricular cartilage grafts survive when placed in an anterior airway defect.[5,11] However, because the revascularization and re-epithelialization processes originate from the margins of the defect, healing of cartilage grafts will depend on the size of the graft. It can be anticipated that the central part of the graft may undergo necrosis if a large patch of cartilage is used. The nature of the mucosal lining at the defect

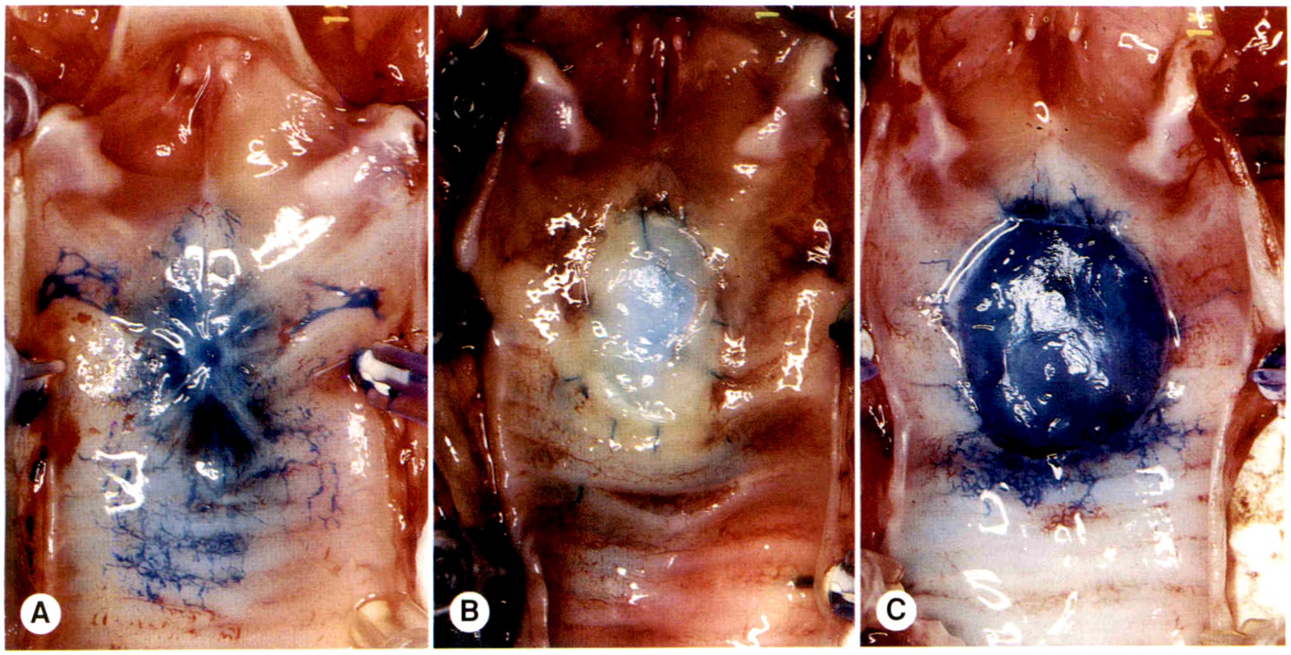

Fig 2. Macroscopy of patch reconstructions. **A)** Macroscopy of fascia. Internal view after posterior longitudinal incision. Fascia is injected with silicone dye (Microfil). Signs of wound contraction, which are most pronounced in center of patch, are visible. **B)** Macroscopy of cartilage. Prolene sutures indicate margins of cartilage graft. Cartilage grafts are remucosalized from margins of defect. In this case, central part of graft was not completely re-epithelialized. **C)** Macroscopy of mucosa-lined fascia. Oral mucosa is completely colored by Microfil. Reconstruction shows primary healing with sharp demarcation line between oral and respiratory epithelial linings.

margins and the degree of vascularization of the defect margins will play an important role in cartilage healing. As a consequence, the healing of cartilage grafts within an airway stenosis may be more difficult because of scarring at the margins of the defect with problematic revascularization and re-epithelialization from these margins.

The vascularized fascia lined with buccal mucosa allows for healing by primary intention. The vascular supply and the epithelial lining of the graft make the patch independent of the defect margins in terms of its healing. The fact that no revascularization and no re-epithelialization are necessary during healing may be advantageous in the clinical situation when a scarred wound bed is encountered. Wound healing with mucosa-lined fascia is comparable to the wound healing seen with skin-lined flaps. A mucosal lining is, however, preferable for airway lining, in order to prevent the crusting and desquamation seen with skin

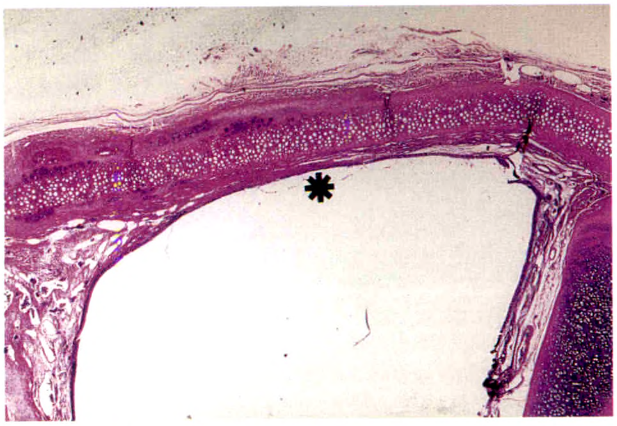

Fig 3. Microscopy of cartilage (H & E, original ×5). Cartilage graft is revascularized and re-epithelialized from margins of defect. Regenerated submucosal blood vessels and respiratory epithelium are more developed at margins of cartilage graft than in middle (asterisk) of graft.

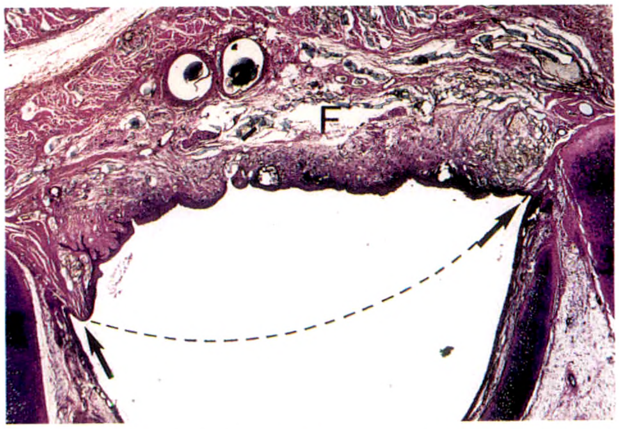

Fig 4. Microscopy of mucosa-lined fascia (H & E, original ×5). Intensely perfused fascia flap (F; blue Microfil–injected) is lined with squamous epithelium. Sharp transition area exists between oral and respiratory mucosae (arrows). Flat reconstruction between margins of defect will be provided when size of mucosal graft is similar to size of defect. Prolapse into lumen will result if bigger patch is sutured into defect (dotted line).

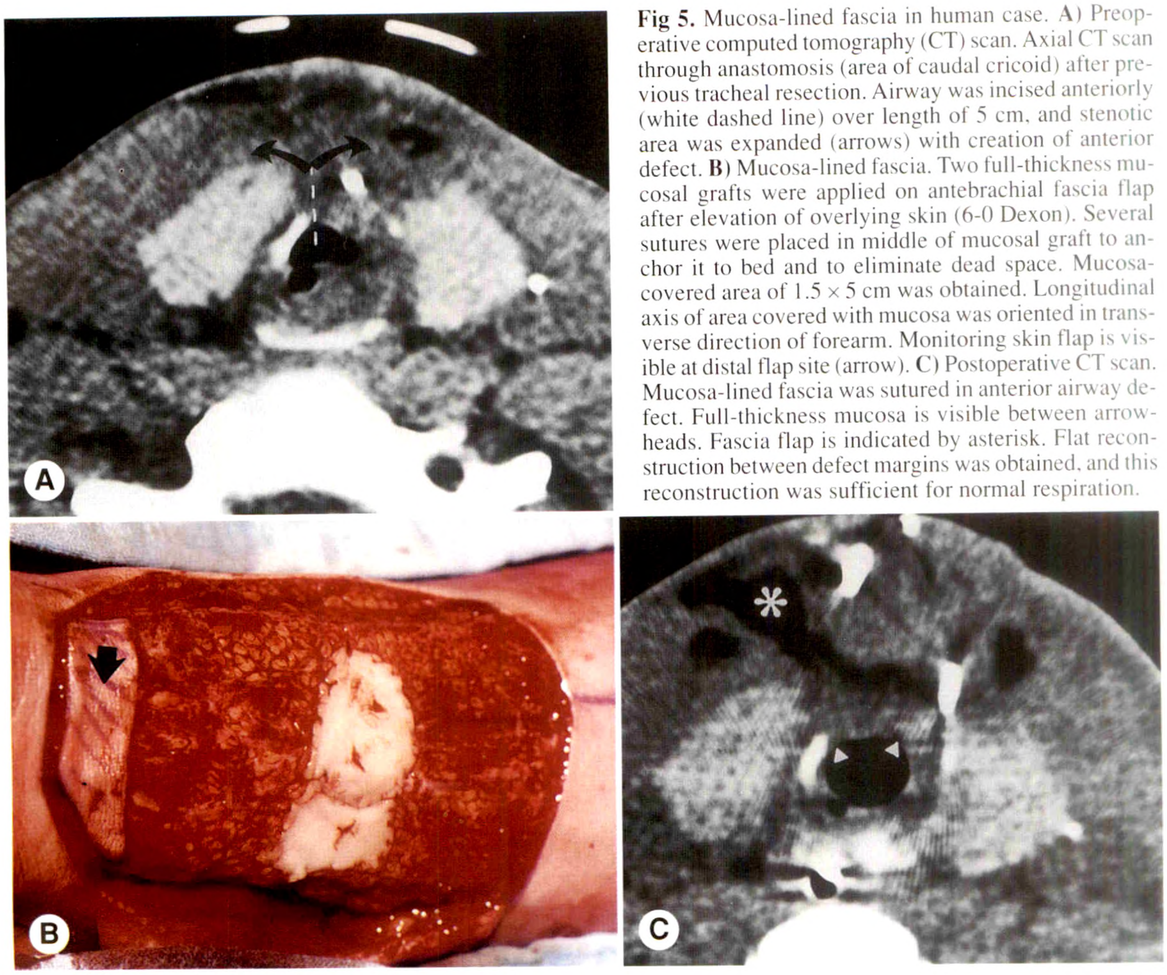

Fig 5. Mucosa-lined fascia in human case. **A)** Preoperative computed tomography (CT) scan. Axial CT scan through anastomosis (area of caudal cricoid) after previous tracheal resection. Airway was incised anteriorly (white dashed line) over length of 5 cm, and stenotic area was expanded (arrows) with creation of anterior defect. **B)** Mucosa-lined fascia. Two full-thickness mucosal grafts were applied on antebrachial fascia flap after elevation of overlying skin (6-0 Dexon). Several sutures were placed in middle of mucosal graft to anchor it to bed and to eliminate dead space. Mucosa-covered area of 1.5 × 5 cm was obtained. Longitudinal axis of area covered with mucosa was oriented in transverse direction of forearm. Monitoring skin flap is visible at distal flap site (arrow). **C)** Postoperative CT scan. Mucosa-lined fascia was sutured in anterior airway defect. Full-thickness mucosa is visible between arrowheads. Fascia flap is indicated by asterisk. Flat reconstruction between defect margins was obtained, and this reconstruction was sufficient for normal respiration.

grafts. A disadvantage of the mucosa-lined fascia is the absence of supportive tissue. It is essential, in introducing a mucosa-lined fascia flap into an airway defect, that the mucosal patch and the defect have a similar size. Prolapse into the airway will result if the mucosal patch is larger than the defect (Fig 4).

Mucosa-lined fascia was used in 2 clinical cases of anastomotic stricture after segmental airway resection.

MUCOSA-LINED FASCIA CASE REPORT

An 18-year-old boy was admitted to our department with severe laryngotracheal stenosis. The stenosis occurred after the patient was involved in a car accident 4 months earlier. At that time, the patient had been intubated for 3 weeks. After extubation, he underwent endoscopy and placement of a tracheotomy tube because of increasing dyspnea. He was found to have subglottic stenosis. The stenosis was initially treated by dilation and laser resection. He was referred to us in October 1997 with a subglottic stenosis with complete obstruction of the lumen (Cotton grade 4) that started 1 cm below the vocal cords. On a computed tomography (CT) scan, the stenotic segment was estimated as having a length of 3 cm. In November 1997, the patient underwent a resection of the cricoid cartilage anteriorly, together with a resection of 4 cm of the proximal trachea including the tracheotomy site. The mediastinal trachea was mobilized, and a suprahyoid release was performed. The mediastinal trachea was sutured to the cricoid cartilage posteriorly and to the thyroid cartilage anteriorly. Although the upper and lower airway segments were mobilized maximally, the anastomosis (2-0 Dexon) was still performed under some tension. The patient was extubated 1 day after the operation, and head flexion was maintained for 1 week. Five weeks after the operation, the patient underwent a replacement of the tracheotomy tube because of increasing dyspnea. On laryngoscopy, a restenosis at the anastomosis was seen, with a 90% reduction of the airway lumen. Two attempts to remove the fibrotic tissue with a CO_2 laser were not successful. In January 1999, an augmentation tracheoplasty with a mucosa-lined fascia flap was decided on. The laryngotracheal complex was incised longitudinally starting at the caudal end of the anterior commissure and ending in the tracheotomy incision. The restenosis was located at the caudal cricoid level and had a length of 2 cm. The laryngotracheal complex was incised over 5 cm

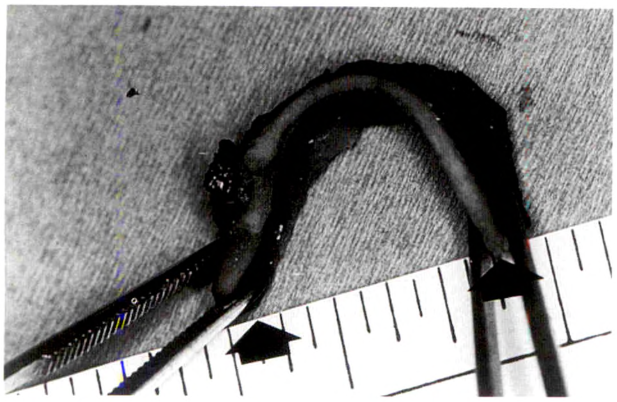

Fig 6. Cartilage support in airway reconstruction for additional lumen augmentation. In humans, auricular graft with dimensions approaching 2 × 5 cm may be harvested. Anterior convexity may be created if elastic cartilage with length larger than width of defect is sutured to margins of that defect (arrows). Scale is in inches.

to allow for a sufficient expansion of the stenotic area. An elliptical anterior defect with a length of 5 cm and a width of 1.8 cm was obtained (Fig 5A). The tracheotomy tube was removed, and the patient was intubated orally.

A radial forearm fascia flap was used as the vascular carrier for the mucosal lining, because it provides fascial and fasciocutaneous tissue, vascularized by a long and reliable vascular pedicle consisting of the radial artery and vein. A patch of fascia and subcutaneous tissue, measuring 8 × 5 cm, was exposed after a midline longitudinal incision of the radial forearm skin. A small strip of skin (measuring 1 × 5 cm) was included distally as a fasciocutaneous segment serving as a monitor for the postoperative viability of the flap (Fig 5B).

Two full-thickness mucosal grafts were harvested at the left and right buccal areas. The buccal mucosa defects were closed primarily. The mucosal grafts were trimmed and cleaned from underlying submucosal fat. The margins of the mucosal graft were secured to the fascia with interrupted sutures. A mucosa-covered area of 1.8 × 5 cm was obtained (6-0 Vicryl).

The grafts were applied before dissection of the flap, because the stability of the undissected fascia allowed for easy suturing. The longitudinal axis of the mucosal graft formed the tranverse axis of the fascia flap (Fig 5B). After the mucosal grafts were applied, the fascia flap was dissected on its vascular pedicle. The skin at the donor defect was closed primarily.

The mucosa-covered area of the fascia flap was sutured into the anterior airway defect with 3-0 Dexon. The distal end of the fascia flap was rotated 180°, and the fasciocutaneous island was brought into the neck incision to serve as a monitor. The monitoring skin paddle was used as a visible control of the viability of the flap in the postoperative period.

After insetting of the mucosa-lined patch, the fascia flap was revascularized by an end-to-end anastomosis between the superior thyroid artery and the radial artery and by an end-to-side anastomosis between the internal jugular vein and the radial vein. The patient was extubated without problems 5 days after the operation. A sufficient airway lumen with the mucosa-lined fascia in the anterior defect was seen on a postoperative CT scan (Fig 5C).

One other patient with a restenosis after segmental resection successfully underwent reconstruction in an identical way. The 2 cases showed that a mucosa-lined radial forearm fascia flap could be used successfully to close the airway defect after longitudinal incision and expansion of a stenotic area. The vascularized fascia allowed for easy healing in a scarred operative field, and the mucosal grafts allowed for primary healing without wound contraction. A disadvantage of the mucosa-lined fascia is the absence of a cartilage component. Because support is lacking, a flat reconstruction between the margins of the anterior defect is the best result that can be obtained. Further expansion of the airway lumen by creating an anterior convexity is not possible, and this may be a drawback when a more extensive stenosis needs reconstruction. In a second set of experiments, we wanted to investigate whether we could improve this reconstructive concept by adding cartilage support to the mucosa-lined fascia flap.

COMPOSITE RECONSTRUCTION MODEL

INTRODUCTION

Cartilage support is essential when a more extended anterior lumen expansion is warranted. This support may be provided if an elastic piece of cartilage is sutured between the margins of an airway defect that is smaller than the cartilage segment. Elastic ear cartilage is available in large quantities in rabbits, but only in small quantities in humans (Fig 6). Fibrocartilage is available in much larger quantities in the human rib, but this type of cartilage has a low elasticity and is not suitable for anterior airway lumen expansion.

Theoretically, 3 different designs are possible when adding elastic cartilaginous support to a mucosa-lined fascia flap. In the first design (composite tissue concept 1), the cartilage graft is placed between inner and outer fascial layers. The full-thickness buccal mucosa graft is applied on the outer fascial layer af-

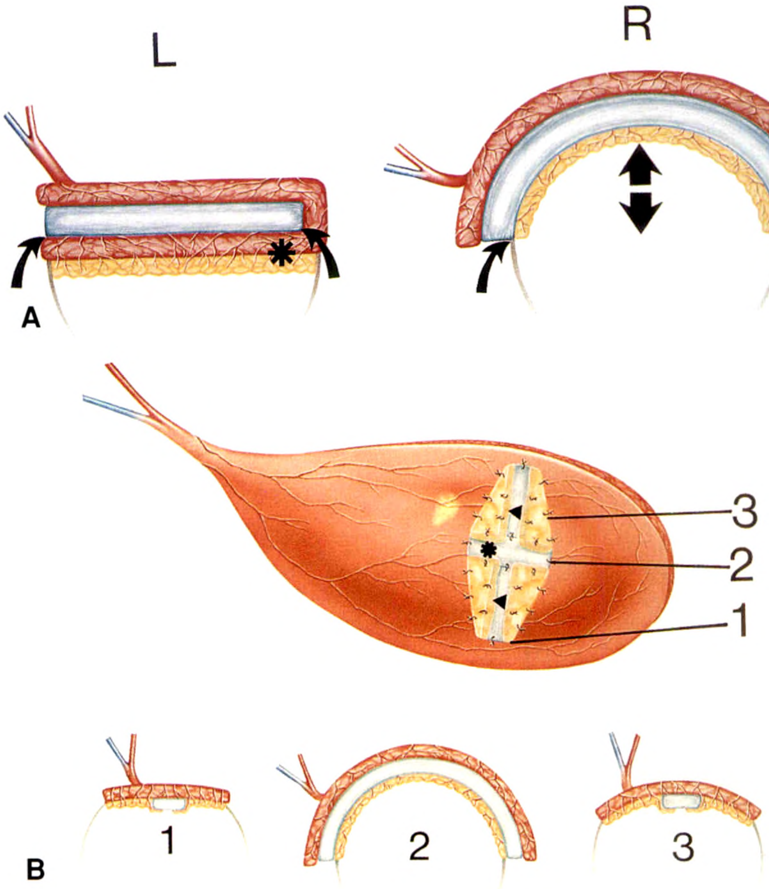

Fig 7. Theoretical possibilities for composite tissue reconstruction. **A)** On left (L) is composite tissue concept 1. Composite tissue is formed by 180° rotation of vascularized fascia. In this way, cartilage is circumferentially covered and protected within vascular bed. Full-thickness mucosal graft can be revascularized by capillary outgrowth from inner layer of vascularized fascia flap. One disadvantage of this concept is low degree of support provided by cartilage component. In order to obtain anterior expansion of airway lumen, margins of cartilage graft (arrows) need to be sutured to margins of defect. In this concept, cartilage margin, which is wrapped with fascia, cannot be sutured to margin of defect without interfering with blood supply to inner fascial layer (asterisk). At right (R) is composite tissue concept 2. Cartilage component is sutured to vascularized fascia and directly attached to mucosal graft. In this concept, margins of cartilage graft (single arrows) may be sutured to margins of defect with anterior expansion of airway lumen (double arrow). Mucosal component will, however, undergo necrosis, because cartilage will not allow vessel ingrowth from fascia to mucosal graft. **B)** Composite tissue concept 3. Cartilage grafts are sutured to fascia flap between mucosal grafts. In this concept, 3 cartilage grafts are sutured to fascial flap. First cartilage graft (asterisk) is sutured to longitudinal axis of flap and will be used for airway lumen expansion. Other 2 cartilage grafts (arrowheads) are applied in transverse axis of fascia flap and give some longitudinal strength to reconstruction. Cartilage grafts are sutured to each other. Four buccal mucosa grafts are sutured to fascia flap and to cartilage grafts to form grafted area that will be used in anterior airway reconstruction. Anterior extension of airway lumen will be obtained at site of cartilage graft (2), while flat reconstruction will be obtained at both ends of composite flap (1). Situation between convex and flat reconstruction will be seen at site between middle and extremity of flap (3). In this concept, cartilage component may undergo secondary healing from 2 sites: from oral mucosa grafts and from respiratory epithelium at margins of defect.

ter 180° rotation of the fascia flap (Fig 7A, left). In this concept, both the cartilaginous and the mucosal components are well perfused by the fascia flap. The problem with this concept, however, is the low degree of support provided by the cartilage component. In order to obtain a convexity at the site of reconstruction, it is necessary to suture the margins of the elastic cartilage graft to the margins of a smaller airway defect. With composite tissue concept 1, the margins of the mucosal graft are sutured to the margins of the defect, and as a consequence, the cartilage graft is located outside the defect without providing support to the reconstruction[12] (Fig 7A, left).

In composite tissue concept 2, the mucosal graft is applied directly to the cartilage graft (Fig 7A, right). This theoretical concept provides cartilage support, but the mucosal graft cannot survive on the cartilage, because the cartilage component will not allow for ingrowth of blood vessels.

In composite tissue concept 3, cartilage strips are applied on the fascia flap so that the mucosal grafts are located within the cartilaginous framework. With this concept, the airway lumen can be expanded at

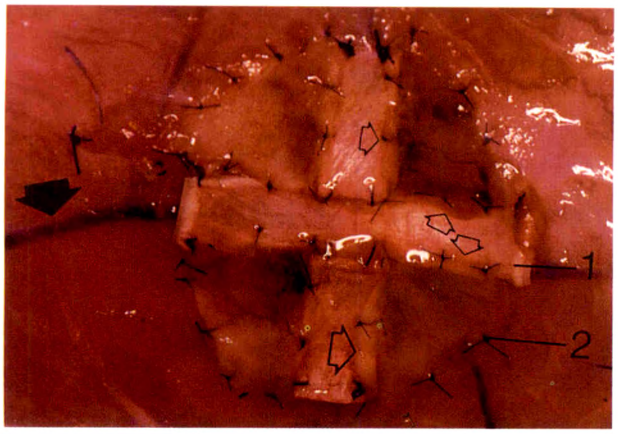

Fig 8. Experimental study of composite tissue design. Situation 2 weeks after grafting of fascia with mucosa and cartilage. Full-thickness mucosal grafts show complete survival. Submucosal vessel ingrowth is visible. Oral mucosa has begun to grow over edges of cartilage grafts (hollow arrows). Solid arrow points to lateral thoracic blood vessels running in axial axis of flap. After follow-up, lateral thoracic artery may be injected with blue Microfil so that blood vessels that are of fascial origin can be identified (see Fig 9). 1 — level of axial section of Fig 9A; 2 — level of axial section of Fig 9B.

the sites at which the cartilage strips are sutured to the margins of the defect (Fig 7B). This concept may give some additional airway lumen expansion by providing cartilage support at the most critical site of the stenosis. Composite tissue concept 3 may theoretically improve the mucosa-lined fascia flap. The healing and amount of support provided by this theoretically most appealing concept were studied in the experimental setting of an anterior airway defect.

ANIMAL STUDY

One-Stage Reconstruction. The healing of the cartilage component of the composite reconstruction was evaluated after repair of an anterior laryngotracheal defect in 3 rabbits. The composite tissue was formed at the lateral thoracic area. Three cartilages and 4 mucosal grafts were sutured to the fascia (5-0 Dexon) according to the pattern shown in Fig 7B. A grafted area of 1.5 × 2 cm was obtained. The composite tissue was transplanted to an anterior laryngotracheal defect measuring 0.8 cm wide and 2 cm long in 3 animals, involving the anterior cricoid cartilage and the first 4 or 5 tracheal rings. The rabbits were followed until the first signs of dyspnea became apparent. At the time of dyspnea, the animals were painlessly killed after injection of the vascular pedicle of the fascia flap with blue Microfil. The reconstructions were assessed macroscopically and histologically.

The 3 animals had normal respiration during the early postoperative days. After an average follow-up time of 5 days, the animals showed signs of progressive dyspnea. Postmortem evaluation of the grafts showed accumulation of secretions over the cartilage grafts. The cartilage grafts showed macroscopic (loss of support) and histologic (eosinophilic ground substance, loss of cartilaginous tissue) signs of necrosis. The oral mucosa grafts showed ingrowth on the fascia flap with early signs of revascularization.

From the early postoperative follow-up in all 3 animals, we learned that the initial support provided by composite tissue concept 3 was lost because of necrosis of the uncovered, airway-exposed cartilage.

Preformed Composite Reconstruction. The principle of flap prefabrication was used to improve the survival of the cartilage grafts. In this technique, transfer of the composite flap was delayed until the

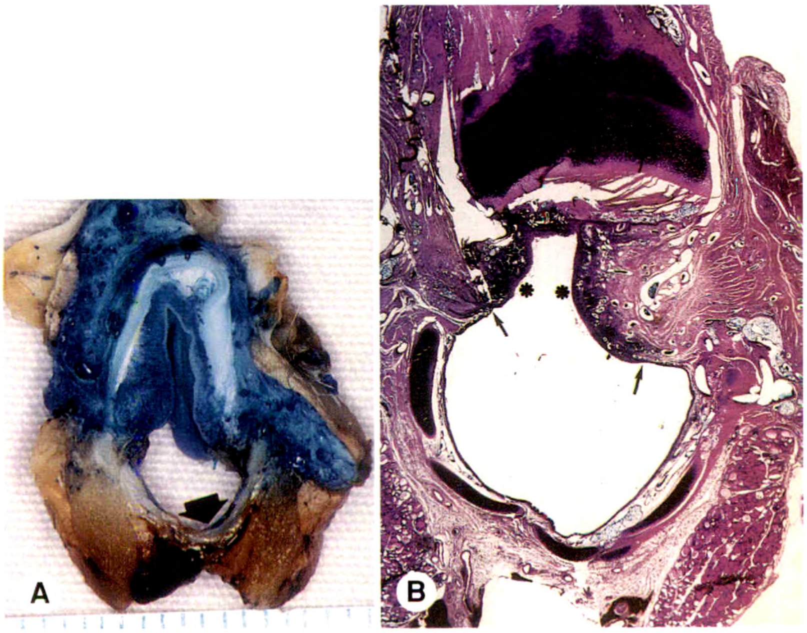

Fig 9. Experimental study of preformed composite tissue. **A)** Macroscopy of axial section at level of cartilage graft applied in transverse axis of reconstruction. Cartilage graft was sutured between margins of anterior defect and expands airway lumen anteriorly. Part of this anterior lumen extension was lost because of secondary healing with buccal mucosa. Mucosal lining is completely vascularized by fascia flap (Microfil-injected). Regenerated buccal mucosa is much thicker than respiratory mucosa that is visible in remaining posterior trachea (arrow). **B)** Histology of axial section at level of mucosal graft. Cartilage strip, which was applied in longitudinal axis of reconstruction, is covered with buccal mucosa. Mucosa-lined fascia is prolapsing into lumen at sites without cartilaginous support (asterisks). Preformed flap, which was sutured into anterior airway defect, is fully covered with buccal mucosa. Sharp demarcation between oral and respiratory epithelia is visible (arrows). Neochondrification is visible on extraluminal surface of cartilage graft.

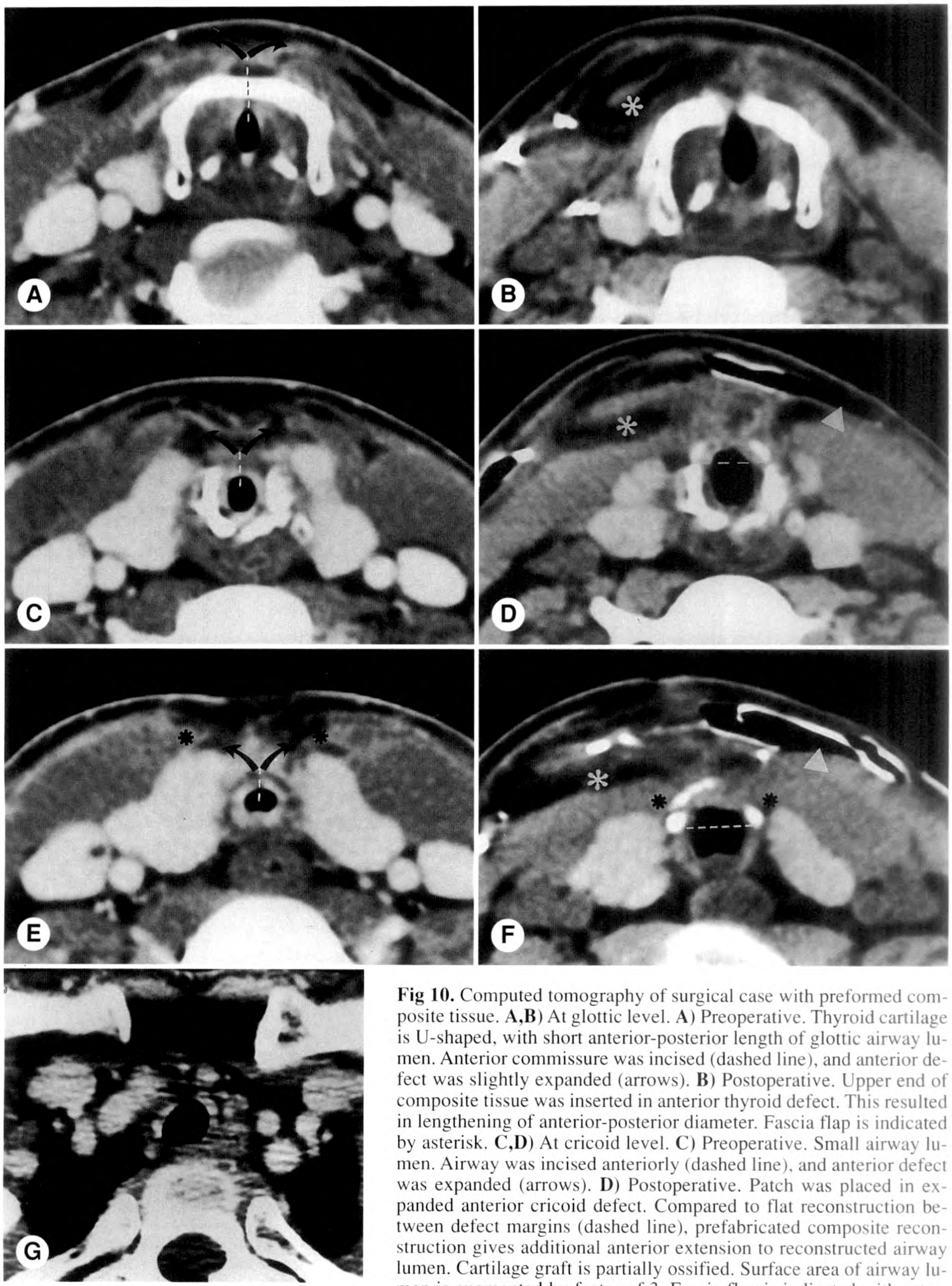

Fig 10. Computed tomography of surgical case with preformed composite tissue. **A,B)** At glottic level. **A)** Preoperative. Thyroid cartilage is U-shaped, with short anterior-posterior length of glottic airway lumen. Anterior commissure was incised (dashed line), and anterior defect was slightly expanded (arrows). **B)** Postoperative. Upper end of composite tissue was inserted in anterior thyroid defect. This resulted in lengthening of anterior-posterior diameter. Fascia flap is indicated by asterisk. **C,D)** At cricoid level. **C)** Preoperative. Small airway lumen. Airway was incised anteriorly (dashed line), and anterior defect was expanded (arrows). **D)** Postoperative. Patch was placed in expanded anterior cricoid defect. Compared to flat reconstruction between defect margins (dashed line), prefabricated composite reconstruction gives additional anterior extension to reconstructed airway lumen. Cartilage graft is partially ossified. Surface area of airway lumen is augmented by factor of 3. Fascia flap is indicated with asterisk. Arrowhead points to postoperative drain. **E-G)** At tracheal level. **E)** Preoperative. Small airway lumen. Airway was incised anteriorly (dashed line), and anterior defect was expanded (arrows). Some sutures (4-0 Prolene) were placed between margins of incised trachea and strap muscles (asterisks) to hold anterior defect in expanded position. **F)** Postoperative. Patch was placed in expanded anterior tracheal defect. Note that strap muscles are displaced to margins of anterior tracheal defect (black asterisks). Compared to flat reconstruction between defect margins (dashed line), prefabricated composite reconstruction gives additional anterior extension to airway lumen. Cartilage graft is partially ossified. Surface area of airway lumen is expanded by factor of 3. White asterisk indicates fascia flap. Arrowhead points to postoperative drain. **G)** Mediastinal trachea. At level of upper mediastinum, airway lumen has normal caliber.

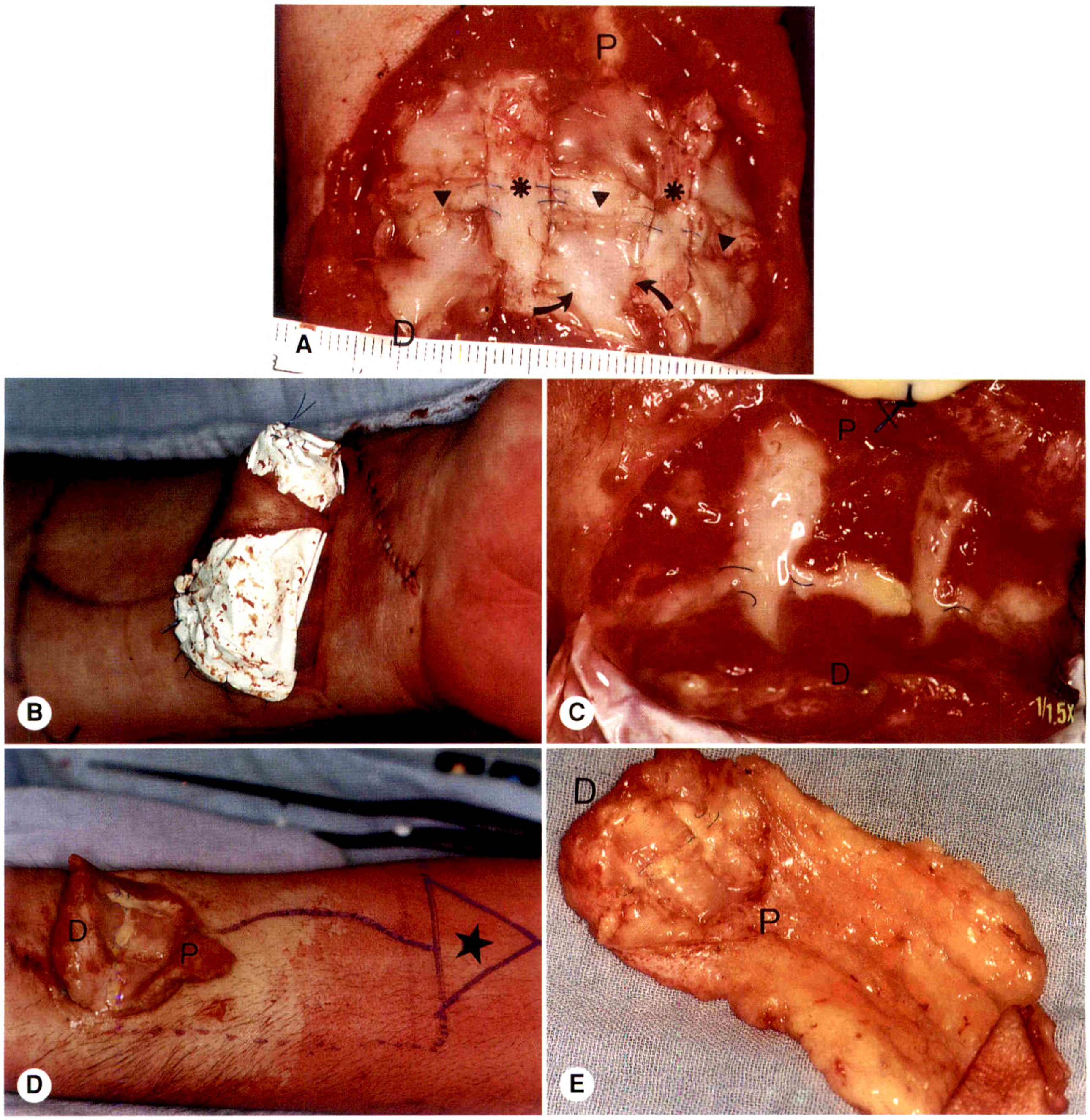

Fig 11. Surgical case with preformed composite tissue. D — distal site of composite tissue; P — proximal site of composite tissue. **A)** Distal antebrachial fascia was grafted with cartilaginous framework. Two strips of auricular cartilage (asterisks) measuring 1 × 4 cm were applied in longitudinal direction of forearm to provide for anterior lumen expansion of reconstruction. Three shorter segments (arrowheads) were applied in transverse direction of forearm in order to give reconstruction some rigidity in longitudinal axis of reconstruction. Cartilage grafts were sutured to fascia (4-0 Dexon) and to each other (4-0 Prolene). Six mucosal grafts (1.2 × 1.2 cm) were sutured within cartilaginous framework with 6-0 Dexon. Patch had length of 5.5 cm (longitudinal axis of reconstruction; transverse axis of forearm) and width of 4 cm (transverse axis of reconstruction; longitudinal axis of forearm). Distal site of preformed composite tissue was wrapped (arrows) around tube to hold cartilage grafts in convex shape during healing. **B)** Waiting period. Composite tissue flap is wrapped around tube. Skin defect cannot be closed, and whole reconstruction is protected with polytef (Gore-Tex) membrane. **C)** After 20 days. Cartilage grafts are covered by oral mucosa at their margins. Note convex shape of composite tissue in longitudinal axis of forearm. Sutures anchoring grafts to fascia flap were removed, except for Prolene sutures between cartilage grafts. **D)** After 20 days, at time of flap dissection. Incision of forearm skin and outlining of monitoring skin paddle (star) are shown. **E)** After flap dissection. Distal part of flap contains composite tissue. Proximal part of fascial flap contains monitoring skin paddle. Intervening tissue consists of vascularized fascia.

cartilage grafts were sufficiently healed and covered with buccal mucosa.

The composite tissue was formed at the lateral thoracic area. Three cartilages and 4 mucosal grafts were sutured to the fascia according to the pattern shown in Fig 8. A grafted area of 1.5 × 2 cm was obtained. This grafted area was wrapped around a Gore-Tex tube to allow for healing of the cartilage grafts in a convex shape. The grafted area was inspected weekly under general anesthesia.

One week after flap prefabrication, the mucosal grafts of the composite reconstruction showed a complete take, and the bare cartilages were visible between the mucosal grafts. After 2 weeks, the peripheral parts of the cartilage grafts showed revascularization and remucosalization through angiogenesis and mucosal overgrowth from the surrounding buccal mucosa grafts (Fig 8). After 4 weeks, the cartilage grafts were almost completely healed and covered by buccal mucosa in all 3 animals. One month after flap prefabrication, the cartilage grafts were sufficiently healed to allow for transplantation to the defect. After secondary healing of the cartilage grafts, the preformed composite tissue was dissected and transplanted to an anterior laryngotracheal defect measuring 0.8 cm wide and 2 cm long in 3 animals, involving the anterior cricoid cartilage and the first 4 or 5 tracheal rings. One month after laryngotracheal reconstruction, the laryngotracheal complex was harvested after injection of the vascular pedicle of the fascia flap with blue Microfil. The reconstructions were evaluated macroscopically and histologically (hematoxylin-eosin staining) on axial sections.

The cartilage grafts were fully healed and completely covered with mucosa in all 3 animals. The preformed composite reconstructions succeeded in expanding the reconstructed airway lumen (length of cartilage strip, 0.7 cm longer than width of anterior airway defect; Fig 9).

The practice of composite tissue prefabrication allowed for secondary mucosal healing of the cartilage grafts before they were exposed to the airway lumen. The principle of prefabrication of the composite tissue was then used in a difficult clinical case of long-segment stenosis.

CLINICAL APPLICATION CASE REPORT

A 38-year-old man presenting with progressive stridor was urgently intubated. He was known to have laryngotracheal hypoplasia with episodes of dyspnea related to exercise. The present condition of extreme dyspnea was triggered by an upper airway infection. Because of the relatively small laryngeal inlet, a pediatric tube was used for intubation. Attempts to extubate the patient proved to be impossible, and a tracheotomy tube was placed after 1 week.

After tracheotomy, the larynx and trachea were evaluated clinically and radiologically. On laryngoscopy, the glottic airway lumen was small, with a short anterior-posterior dimension. Vocal fold mobility was preserved, without signs of arytenoid ankylosis. A small airway lumen was found from the glottic level to the level of the tracheotomy. The length of the laryngotracheal stenosis measured 5.5 cm. The laryngeal cartilages had an abnormal form and shape, and they were intensely calcified (Fig 10A). The mediastinal and thoracic parts of the trachea had a normal airway lumen (Fig 10G). For removal of the tracheotomy tube, a tracheoplasty over a length of 5.5 cm was necessary. This intervention was also necessary for the patient to regain the ability to speak. The patient could not speak with the cannula because of a complete obstruction of the airway lumen above the tracheotomy site. Segmental airway resection or a slide tracheoplasty seemed impossible in this case because of involvement at the glottic level.

In the first operation stage, full-thickness mucosal grafts from the right and left buccal areas, as well as auricular cartilage taken from both ears, were grafted to the left radial forearm fascia. Two auricular cartilages measuring 2 × 4 cm were obtained. Two cartilage grafts of 1 × 4 cm were sutured (4-0 Dexon) to the longitudinal axis of the fascia to allow for anterior expansion of the reconstructed airway lumen (Fig 11A). Three cartilage grafts were sutured to the tranverse axis of the flap. The cartilage grafts were sutured to each other with 4-0 Prolene. Six full-thickness mucosal grafts were applied within the cartilaginous framework. A fascial area of 5.5 × 4 cm, grafted with mucosa and cartilage, was obtained after suturing the mucosal grafts to the fascia (4-0 Dexon). The grafted fascial area was sutured around a Gore-Tex tube to allow for secondary healing of the cartilage grafts in a convex shape. The forearm skin could not be closed over the preformed tissue, and a Gore-Tex patch with paraffin gauze dressing was used to cover the tissue (Fig 11B). The preformed tissue was inspected 10 days later under general anesthesia. The Gore-Tex was then removed, and the composite tissue was inspected. The sutures anchoring the grafts to the fascia were removed, except for the Prolene sutures between the different cartilage strips. A progressive growth of blood vessels and buccal mucosa over the cartilage grafts could be seen.

It was decided to transplant the preformed tissue 20 days after the initial operation. At that time, the cartilage strips were not completely re-epithelialized, but they were sufficiently healed at the margins of the mucosal grafts to allow for transplantation (Fig 11C). The forearm fascia was dissected on its vascular pedicle (Fig 11D,E). The neck was opened, and the larynx and trachea were incised longitudinally from the level of the vocal folds to the level of the mediastinal trachea over a length of 5.5 cm. The anterior airway defect was expanded. The margins of the incised trachea were sutured to the strap muscles in order to maintain the expanded position of the incised anterior airway defect (Fig 10C-F). The tracheotomy tube was removed, and the patient was intubated orally. The composite flap was sutured in the anterior airway defect with 3-0 Dexon.

The flap was revascularized by suturing of the radial vessels to the superior thyroid artery (end-to-end) and to the internal jugular vein (end-to-side). A fasciocutaneous portion was included at the proximal site of the flap and sutured in the neck incision to serve as a monitor.

The patient was extubated without problems 5 days after the operation. A CT evaluation performed 10 days after the operation showed a laryngotracheal airway lumen that was expanded by a factor of 3. The cartilage support was preserved, and the reconstructed airway had a convex shape anteriorly (Fig 10).

DISCUSSION

Cartilage grafts are frequently used in laryngotracheal reconstruction.[5] They are the optimal tissue to place between the divided halves of the cricoid cartilage to resolve a posterior laryngeal stenosis. Their use has become less popular in the anterior larynx and trachea, because better results may be obtained with resection and end-to-end anastomosis.[1,2] A disadvantage when using free cartilage is that the revascularization and re-epithelialization processes originate from the margins of the defect. The marginal revascularization and re-epithelialization may be sufficient for small grafts in a well-vascularized wound bed, but will be jeopardized in cases of scarring or with large strips of cartilage.

Mucosa-lined fascia has the advantages of primary healing and of a good intrinsic vascularity. This tissue may be used to resolve restenosis after segmental resection. It can be used in a 1-stage procedure with good survival of the full-thickness mucosal grafts. A prerequisite for mucosal survival is that the margins and the central portion of the grafts are carefully sutured to the vascularized fascia.

Composite tissue consisting of mucosal lining and strips of cartilage is the most adequate tissue that is currently available for airway wall reconstruction. This tissue can be used to repair critical airway segments. Flap prefabrication needs to be done to allow for survival of the cartilage component. Overgrowth of the bare cartilage by the mucosal lining, which is essential for cartilage survival, is a slow process. It took 1 month to obtain a complete coverage of the cartilage grafts (0.3 cm wide) in the rabbit model. In the clinical case, relining of the cartilage grafts (1 cm wide) was still incomplete after 20 days. Because of the slow remucosalization, the cartilage strips may undergo necrosis when they are immediately exposed to the airway lumen during a 1-stage procedure. After flap prefabrication, the cartilage grafts are sufficiently healed so that the cartilage support remains preserved.

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THE DFNA10 PHENOTYPE

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We present a detailed analysis of the DFNA10 phenotype based on data from 25 hearing-impaired persons coming from a large American pedigree segregating for deafness at the DFNA10 locus (chromosome 6q22.3-23.2). Cross-sectional analysis of air conduction threshold–on–age data from all available last-visit audiograms (linear regression analysis, age over 15 years) showed progression of hearing loss at a rate of 0.6 dB/y over all frequencies, with a flat to gently sloping age-corrected threshold of about 50 dB. The results were significant at 0.25, 4, and 8 kHz, but only if corrections for presbycusis were not included. Longitudinal threshold analysis performed in 1 case, covering ages 6 to 32 years, showed progression of hearing loss at a rate of 2 to 3 dB/y over 0.25 to 8 kHz. Nonlinear regression analysis was performed on phoneme discrimination scores with use of sigmoidal dose-response curves with variable slope. On the basis of these data, the hearing loss phenotype in this American DFNA10 family can be described as postlingual, initially progressive, and resulting, without the influence of presbycusis, in largely stable, flat sensorineural deafness.

KEY WORDS — autosomal dominant nonsyndromic hearing loss, DFNA10, speech discrimination.

INTRODUCTION

Genetic linkage techniques have facilitated the identification of genes essential for normal auditory function. The initial step in this process is the localization of relevant genes by classic linkage analysis. Since 1992, 39 loci for autosomal dominant nonsyndromic sensorineural hearing loss have been mapped, and 11 of the relevant genes have been cloned. An update of these genetic data can be obtained by consulting the Hereditary Hearing Loss Homepage.[1]

The identification of these genes has been facilitated by detailed descriptions of the hearing loss phenotype. The value of these clinical data is paramount, as the inability to recognize phenocopies in linkage analysis precludes a successful outcome. In addition, with detailed audiometric data, possible phenotypic-genotypic correlations can be explored.

In 1996, the DFNA10 locus was mapped to chromosome 6q22.3-23.2 in an American family.[2] (The "DFN" stands for deafness, the "A" for autosomal dominant, and the "10" for the 10th autosomal dominant locus discovered.) Additional fieldwork has enabled us to expand the pedigree to include more than 200 persons. In this article, we provide a detailed description of the deafness phenotype that is segregating in this family.

PATIENTS AND METHODS

Subjects in the DFNA10 American kindred were identified with the assistance of a family member who maintained a detailed archive of the pedigree that covered approximately 2 centuries (Fig 1). Numerous family branches were identified in which hearing loss was segregating. In these branches, both affected and nonaffected persons were contacted.

In 27 affected persons, pure tone and speech audiograms were obtained by standard procedures; bone conduction levels were evaluated only to exclude conductive loss. Thirteen persons also had tympanograms and acoustic middle ear reflex measurements; 5 persons had serial audiometry. By historical data and physical examination, there was no evidence to suggest that the hearing loss was syndromic. Each affected person also shared a common genotype within the DFNA10 interval.[2] Two persons were suspected of having additional nonhereditary hearing problems and were excluded from the audiological analysis.

Audiometric configuration and threshold asymmetry were evaluated according to the criteria and classification established by the European Work Group

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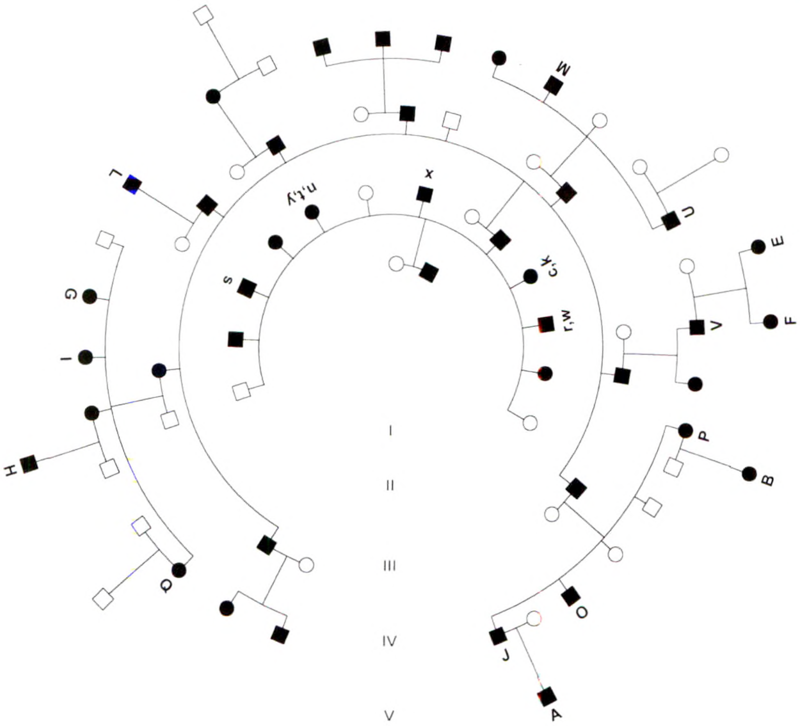

Fig 1. Simplified pedigree of American DFNA10 family. Only 1 branch is shown in full. Participants are indicated by letters. (Descendants are indicated by lowercase letters.) Filled symbols not accompanied by letter indicate persons whose histories indicated hearing loss.

on Genetics of Hearing Impairment.[3] Air conduction thresholds (decibels hearing level [HL], last visit) were used for cross-sectional linear regression analysis (threshold-on-age; age range, 15 to 87 years) with a commercial program (Prism 2.01, GraphPad, San Diego, California). Where relevant, out-of-scale measurements were included and fixed at an arbitrary level of 130 dB HL. The threshold at each frequency was tested for significant progression (regression coefficient of more than 0). The Prism program's F test option for comparing regression lines was used to identify lines that could be pooled.

To detect progression beyond presbycusis, analyses were repeated with corrected values (measured thresholds minus age- and sex-adjusted median thresholds according to ISO 7029 norms[4]). Two persons more than 70 years old were excluded, as there are no presbycusis norms available for their age. In the absence of significant progression, we constructed a "mean audiogram" by compiling all age-corrected threshold data. One-way analysis of variance was used to detect significant differences between thresholds at each frequency. Pooling was performed only where permitted according to the results of such tests.

Longitudinal linear regression analysis (threshold-on-age) was performed in 3 patients. Cross-sectional (last visit) phoneme discrimination scores (phonetically balanced word test; PB-50) were analyzed in relation to age and pure tone average of the thresholds at 1, 2, and 4 kHz. Pure tone average levels were included only if at least 2 of the 3 frequencies involved had been measured. Nonlinear regression analysis was performed with a sigmoidal response curve with variable slope.[5] One-way analysis of variance and Student's *t*-test (with Welch's correction if Bartlett's test demonstrated significantly unequal variances) were used to compare results with those we obtained in a group of 46 controls with only presbycusis (unpublished observations).

RESULTS

All of the hearing-impaired persons dated their first symptoms of hearing loss within the first 3 decades of life. Apart from episodic vertigo with temporary imbalance in 1, none experienced additional relevant symptoms. As is typical of persons with other types of postlingual hearing impairment, all also had normal speech and language development.

Audiograms are shown in Fig 2 (cases A through Y). As only 3 persons had substantial intra-aural threshold asymmetry (not shown), only data pertaining to the right ear were analyzed. The bone conduction thresholds and tympanograms (data not shown) were consistent with the pure tone threshold data. The acoustic middle ear reflexes were normal, assuming a maximum of 100 dB.

Almost 40% of the subjects (cases C, L, N, R, and U through Y) showed steeply sloping audiograms. This configuration was present in 8 of the 14 persons more than 50 years old (cases L, N, R, and U through Y), but in only 1 of the 11 younger persons — a significantly different prevalence (Fisher's ex-

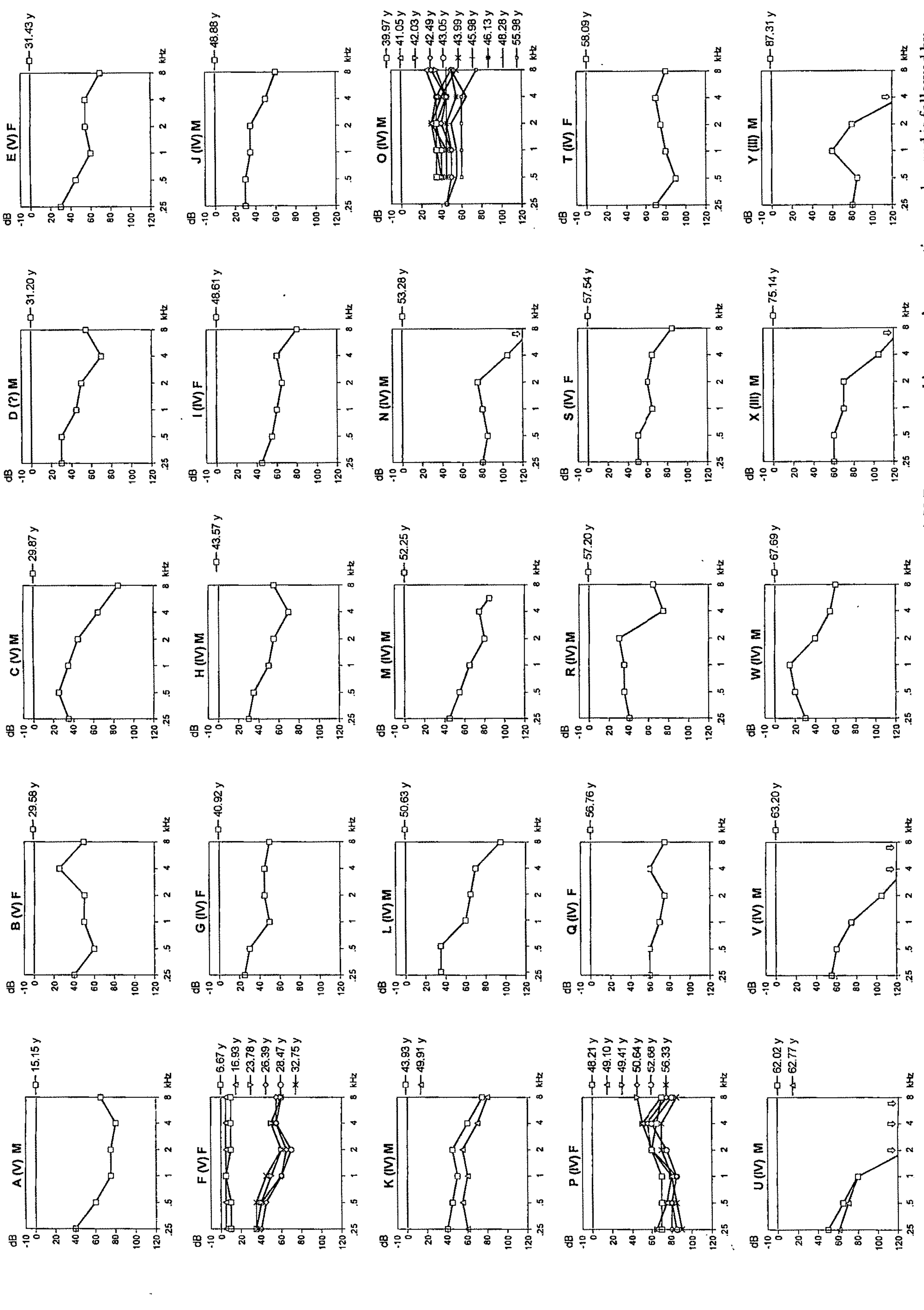

Fig 2. Audiograms (air conduction threshold in decibels hearing level in right ear) in cases A through Y. Roman numeral is person's generation number and is followed by sex (F, M). Note that audiograms are ordered by age at last visit.

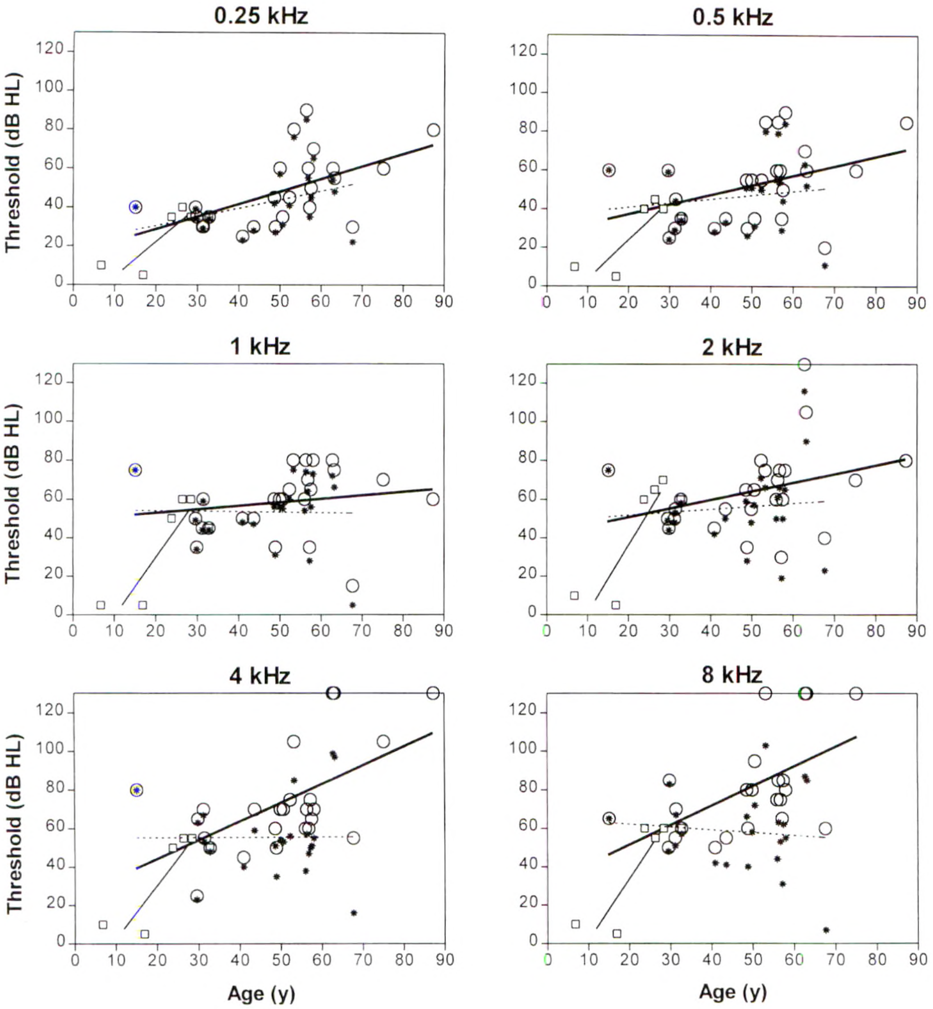

Fig 3. Cross-sectional threshold-on-age data (air conduction threshold in right ear). Circles and bold solid lines relate to linear regression analysis in all cases (age over 15 years). Asterisks and dotted lines relate to age-corrected thresholds (see Methods). In addition, case F is shown separately with squares and thin solid lines.

act probability test, p = .017). Seven persons had a gently sloping configuration (cases D, F, H, J, K, M, and S; ages 31 to 57 years). In 5 persons (cases A, E, G, I, and P), the configuration could not be clearly classified, as it was intermediate between the flat and gently sloping types.[3] Four persons (cases B, O, Q, and T; ages 29 to 58 years) had flat pure tone audiograms.

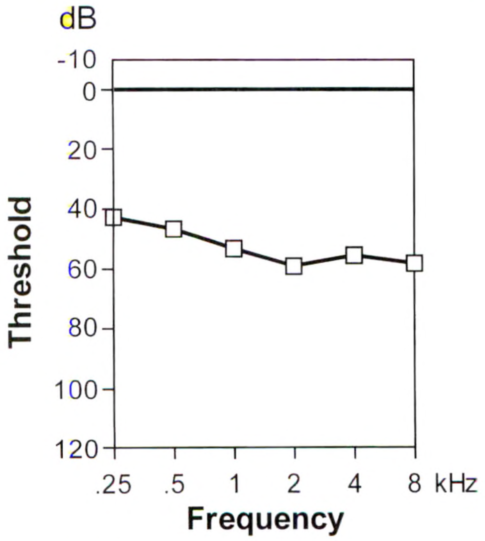

Fig 4. Mean, age-corrected audiogram.

Of the 5 persons with serial audiometry (F, K, O, P, and U), 3 (F, O, and P) had a sufficient number of audiograms covering a sufficiently wide age interval for longitudinal analysis. Both cases O and P showed progression of about 2 dB/y, which was significant only in case O. The longitudinal analysis of case F is combined in Fig 3 with the overall cross-sectional linear regression analysis. This Figure shows a sharp increase in threshold in the second and third decades of life (ie, of case F). The thin trend line connects the data points (squares) obtained by separately averaging the threshold and age values for the first 2 and the last 4 measurements. The derived mean threshold deterioration was approximately 2 to 3 dB/y at 0.25 to 8 kHz. Between the 2 "baseline" measurements (at ages 6 and 16; also see Fig 2), a considerable number of audiograms were obtained from case F, because she had middle ear effusions; the data from this interval have been excluded from the analyses.

The cross-sectional analysis (circles and bold solid

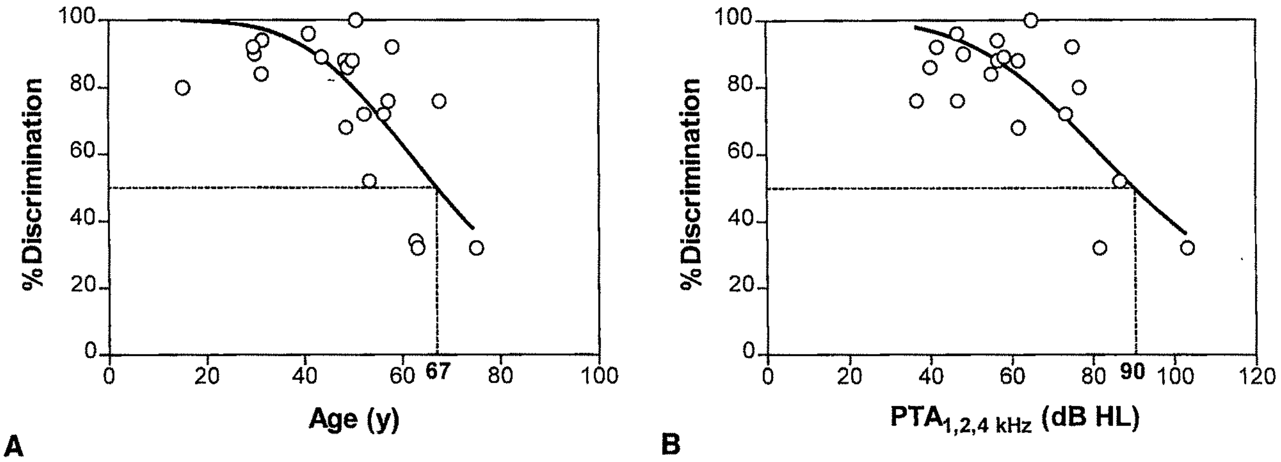

Fig 5. Plot of phoneme discrimination scores in right ear against **A)** age and **B)** corresponding pure tone average of thresholds at 1, 2, and 4 kHz ($PTA_{1,2,4\ kHz}$). Solid lines were fitted with sigmoidal dose-response equation with variable slope; dotted lines indicate inflection point (50% score).

lines) disclosed significant progression at 0.25, 4, and 8 kHz (Fig 3). The annual threshold deterioration pooled for all frequencies was approximately 0.6 dB/y. After correction was made for presbycusis (asterisks and dotted lines), the significance of progression vanished; the pooled annual threshold deterioration was only about 0.1 dB/y. Thus, we concluded that there was no substantial progression beyond presbycusis between ages 30 and 70 years. Case A (age 15 years) showed considerable asymmetry; exceptionally high thresholds were only seen in the right ear. The age-corrected thresholds of all individuals included in the cross-sectional analysis are incorporated in the "mean audiogram" (mean age, 47.6 years; Fig 4).

Figure 5 shows the development of phoneme discrimination in relation to age and PTA level. The 50% score was attained at age 67 years, whereas in the presbycusis group, this score was attained at 89 years (significantly different; standard errors not shown). When the score was plotted against the pure tone average, the sigmoidal curves in the groups of DFNA10 and presbycusis patients were fairly similar: the 50% value was found at 90 dB in the DFNA10 group and at 86 dB in the presbycusis group.

DISCUSSION

The hearing loss phenotype in this American DFNA-10 family is postlingual, progressing to a largely stable flat sensorineural hearing loss on which presbycusis-associated changes can be superimposed. Admittedly, in the absence of enough data in the 0- to 10-year age range, there is only circumstantial evidence for the postlingual onset. However, longitudinal analysis of case F is consistent with hearing acuity that is normal in childhood but begins to deteriorate relatively early. After the age of 30 years, the loss becomes stable, apart from decrements due to presbycusis.

The progressive sensorineural deafness in persons affected by DFNA10 initially occurs without loss of speech discrimination; however, speech discrimination drops as presbycusis aggravates the loss. When thresholds go beyond 50 dB across frequencies, deterioration in speech discrimination is evident. Although this deterioration occurs at a younger age than usual, the rate of deterioration is consistent with the added effects of presbycusis. When we fit parabolas of the form $Y = \text{Offset} + \text{Acceleration}(\text{Age} - 18)^2$ to the threshold-on-age data of DFNA10-affected persons (based on equations in the ISO 7029 norm for presbycusis[4]), at each frequency, Offset is fairly similar to values shown in the mean audiogram (data not shown). From that level onward, thresholds increase along the parabolic curves with the person's age approximately in parallel with classic presbycusis parabolas.

This observation means that the decline in hearing threshold with advancing age in DFNA10-affected persons is a combination of a stationary onset level attained during the first few decades of life (the inherited DFNA10 effect) and normal presbycusis. After the age of 30 years, the hearing loss shows no progression of significance other than what would be expected from presbycusis. Persons with DFNA10 and presbycusis show similar abilities to discriminate phonemes in relation to the pure tone threshold levels. This finding suggests that DFNA10 is like presbycusis in the sense that it is also a primary cochlear dysfunction. The progressive but self-limiting nature of this loss is intriguing.

It is possible that premature outer hair cell damage is the basis for the DFNA10 phenotype, as dysfunction at this level would result in thresholds similar to those we have observed in this family. Once the DFNA10 gene has been cloned, in situ hybridization experiments can be performed to determine where transcripts localize within the cochlea. These data, and the clinical results presented here, will improve our understanding of deafness at the DFNA10 locus and provide us with a clearer picture of normal inner ear function.

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ADDENDUM

Recently, the DFNA10 gene was identified as *EYA4* (Wayne S, Robertson NG, Declau F, et al. Mutations in the transcriptional activator *EYA4* cause late-onset deafness at the DFNA10 locus. Hum Mol Genet 2001;10:195-200).

Ann Otol Rhinol Laryngol 110:2001

MEASUREMENTS OF DRILL-INDUCED TEMPERATURE CHANGE IN THE FACIAL NERVE DURING MASTOID SURGERY: A CADAVERIC MODEL USING DIAMOND BURS

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The purpose of this study was to investigate the changes in temperature in the facial nerve that occur during mastoid drilling by the facial recess approach and to confirm the beneficial effects of constant irrigation while drilling. Mastoid drilling was performed on human cadaveric temporal bones by means of 4-mm diamond burs with and without irrigation. There were 6 subjects in each group. Changes in facial nerve temperature were recorded on a continuous-output monitor. Significant changes were found in temperature for both the irrigation and non-irrigation groups ($p < .0001$). Constant irrigation minimized the rise in temperature. Facial recess drilling poses a potential threat to cranial nerve VII via thermal injury. The temperature elevation in the non-irrigation group was significantly greater than that in the irrigation group and was greater than the tolerable limits of peripheral nervous tissue. This finding supports the need for constant irrigation during the approach to the facial recess in mastoid surgery.

KEY WORDS — facial nerve injury, mastoid drilling, thermal injury.

INTRODUCTION

Injury to the facial nerve occurs most commonly as a result of temporal bone trauma, from either blunt trauma or penetrating wounds. However, the facial nerve is also at risk for injury during middle ear or mastoid surgery. Iatrogenic injury to the facial nerve resulting in paralysis has been reported to occur in 0.6% to 3.6% of otologic surgical procedures.[1] The type of injury varies, including blunt, traction, chemical, or thermal. The percentages of each type of injury have not been reported, partly because it is difficult to determine the cause of injury after surgery. The operating microscope has dramatically reduced the risks of injury, but surgeons still must maintain a heightened awareness of these possible complications, as many injuries are not visually detected at the time of surgery.

Many otologists agree that thermal injury to the facial nerve during mastoid drilling procedures is possible, but no studies have demonstrated actual thermal injury to the nerve. However, 1 study has addressed the magnitude of thermal change that cutting and diamond burs produce during mastoid drilling.[2] The data from this study indicated that thermogenesis varies according to bur design, size, applied pressure, and rotation speed and stated a need for continuous irrigation to prevent thermal injury to bone and nerve tissue. However, it did not investigate whether the temperature in the fallopian canal increased significantly.

Heat damages nerve tissue by protein coagulation and/or inactivation of enzyme systems.[3] In mammals, nerve function has been shown to be permanently impaired by temperatures of 41°C to 45°C and destroyed by temperatures above 45°C.[4,5] The time of exposure to supranormal temperatures may also play a role in thermal nerve injury, as has been shown to occur in a porcine skin experimental model.[6] The change in temperature in bone at a distance of 1 to 2 mm from surface drilling has been shown to be higher with diamond burs than with cutting burs. The temperatures produced are high enough to induce irreversible damage to nerve tissue if no irrigation is used.[2]

This study set out to investigate the temperature change caused in the facial nerve during mastoid drilling on cadaveric temporal bones by means of diamond burs with and without irrigation. The purpose was to determine whether the drill-induced temperature change produced during mastoid drilling procedures is large enough to place the facial nerve at risk for thermal injury, and to confirm the beneficial effect of constant irrigation while drilling.

MATERIALS AND METHODS

The extent of temperature elevation in the mastoid segment of the fallopian canal produced by diamond burs was measured at high rotation speeds, with and without continuous saline irrigation maintained at room temperature. The drilling was performed on 6 dry human cadaveric temporal bones in each of the two groups. The time and temperature changes were simultaneously monitored. All procedures were per-

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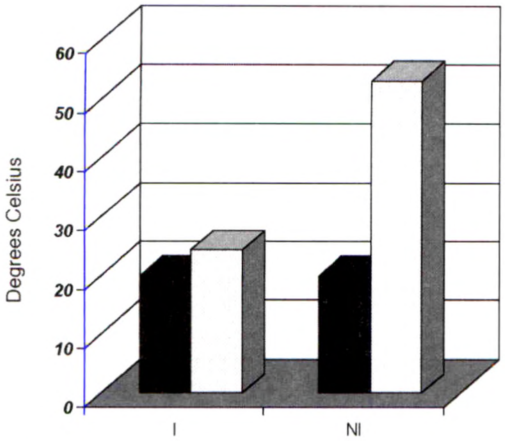

Initial (dark bars) and maximum (light bars) mean temperatures of fallopian canal during drilling procedures in irrigation (I) and non-irrigation (NI) groups. Rise in temperature in NI group was significantly higher than rise in temperature in I group.

formed by the same surgeon (G.M.A.).

The temporal bone drilling was performed with a handheld Smith & Nephew–Richards (Memphis, Tennessee) Synergy Drill. The drill was used at its highest rotation speed (40,000 rpm). The drill times and applied pressures were similar in all bones, but were not controlled. Before any temperature measurements were made, a mastoidectomy was performed to remove most of the air cells overlying the facial recess, the area of experimental interest.

The landmarks used for facial recess drilling and temperature measurement were the incus in the attic, providing a superior margin; the chorda tympani as it traverses from the facial nerve to the middle ear, providing anterior and inferior margins; and the posterior edge of the segment of facial nerve proximal to the takeoff of the chorda tympani, providing a posterior margin. The bones were maintained at room temperature. Before each bone was used for the experiment, a standardized thermistor metal temperature probe (YSI 500 series, Harvard Apparatus, Holliston, Massachusetts) was inserted through the stylomastoid foramen and advanced to the junction of the horizontal and vertical portions of the facial nerve. The temperatures of the temporal bone and probe were allowed to stabilize before starting the procedure. A Cole-Parmer (Vernon Hills, Illinois) Digi-Sense thermistor thermometer was used to continuously record the temperature at 3-second intervals.

A segment approximately 1 cm in length was drilled over the facial recess until the nerve was exposed and the middle ear space was visible. The approximate drilling time for each group was 2 minutes. A new diamond bur was used for each subject. For the irrigation (I) group, normal saline was dripped directly onto the bur during drilling at a constant irrigation rate of 35 mL/min. This was accomplished by the built-in irrigation pump on the Smith & Nephew–Richards Synergy Drill. Constant suctioning was performed in both groups, to remove bone dust from the non-irrigation (NI) group and to prevent flooding of the surgical field in the I group. By maintaining the bones and irrigation at room temperature, we were able to attribute any observed heating to the drilling procedure. A 1-way analysis of variance and Fisher's exact probability tests were used to determine significant group mean differences; $p < .05$ was used as the criterion for statistical significance.

RESULTS

As expected, the heat rise in the area of the facial recess was greatest when drilling was performed without irrigation (see Figure). The mean elevation in temperature was 33°C in the absence of irrigation. However, there was a notable increase in temperature in both groups. There was a mean temperature elevation of 4°C even in the I group. Log transformations of the temperatures for both the I and the NI groups were used for statistical analysis in order to normalize the data. Analysis of variance determined that there was a significant difference ($p < .0001$) between the two groups. Analysis of simple effects was used to demonstrate that there was no difference ($p = .9252$) between the initial temperatures of the two groups, and that there was a significant difference ($p < .0001$) between the maximum temperatures of the two groups.

As explained above, time and pressure were two variables that were not controlled during the drilling procedure. However, time measurements were made for each subject, and statistical analysis with the unpaired *t*-test demonstrated that there was no difference between the drill times for each group ($p = .1461$). Given this result, drill time and pressure were not believed to be responsible for the variability in the temperature measurements.

Finally, on Fisher's exact probability test, there was a significant difference between the groups in temperature elevation of more than 8°C ($p = .0022$). None of the temperature elevations reached this value in the I group, whereas all of the subjects in the NI group had elevations far above this value.

DISCUSSION

Induction of facial paresis by overheating the bone surrounding the facial nerve is a recognized hazard of temporal bone surgery. The thermal injury can occur by protein coagulation and/or inactivation of enzymes in nerve tissue.[3] Shambaugh[7] proposed that this problem could be avoided by using intermittent, rather than continuous, pressure in one spot, but most otologic surgeons do not rely upon this exclusively. A variety of irrigation techniques evolved out of the need to clear the surgical field of bone dust and to prevent clogging of burs. The cooling value of irrigation was initially a secondary issue, with no specific study showing the effectiveness of constant irrigation when using diamond burs in approaching the facial recess. Call's[2] study addressed the use of different types of mastoid burs. His investigation helped to further define the mechanical consideration of heating with different burs, but was limited in several respects. Bone temperature was not constantly monitored, the drilling was not within the mastoid bone, and surgical techniques were not practiced. It has been suggested that the actual temperature gradient is reduced by 3 factors. One factor involves the effect of a constant repositioning of the bur on the temporal bone. A second factor includes the energy used in breaking bone chips and heating of the bur. The third, and most important, factor concerns the loss of heat to the surroundings, especially when irrigation is used.

This study set out to confirm the effectiveness of irrigation during facial recess drilling with diamond burs. The results of this study support the need for constant irrigation during drilling at the facial recess. Although there was a significant rise in temperature for both the I and NI groups, irrigation limited the rise in temperature of the facial nerve.

While studying the thermosensitivity of mammalian A and C nerve fibers, Lundberg[5] observed depolarization following warming to 45°C for 15 seconds. Permanent changes were thought likely if warming continued for a longer duration. Experience with ultrasonic irradiation of the labyrinth for Meniere's disease led Angell et al[8] to the discovery that elevations in temperature greater than 9°C resulted in facial nerve damage. Furthermore, Anderson et al[9] found that temperature elevation in the sciatic nerve between 11.5°C and 14.6°C produced cessation of excitability and complete loss of function if sustained for 2 to 8 minutes. Since the temperature elevations in the NI group in the present study exceeded those noted by the above authors, the facial nerve was likely at risk for irreversible damage.

Since the thermal tolerance of the facial nerve has not been well defined and no information exists regarding return of function to the damaged nerve, surgical techniques must rely on the available information. For the purposes of this study, temperature elevations of 8°C or higher are considered to place the facial nerve at risk for irreversible damage. Of note, there were no recorded temperature elevations of 8°C or higher for the I group; however, every subject in the NI group had elevations greater than 8°C, with a mean increase of 33°C. In fact, the average time spent at a temperature increase of 8°C or higher during drilling for the NI group was more than 70% of the total drilling time. This compelling finding further supports the need for constant irrigation.

It must be recognized that there are limitations to this study. There are multiple variables that may influence the potential for heating, which include movement of the bur over the surgical field, pressure applied during drilling, bur design and sharpness, and irrigant flow rate. These variables were controlled as much as possible by using a constant irrigation rate and a new bur for each subject. The pressure and movement of the bur over the surgical field are operator-dependent, and thus, variance was minimized by having one person perform all of the procedures. A second limitation is that this study does not measure in vivo conditions, in which blood flow may mitigate temperature changes to some extent. The threat of thermal injury to the facial nerve during mastoid drilling procedures has been demonstrated. Further studies addressing histologic and functional changes should be performed in an animal model.

CONCLUSION

This study on the drill-induced temperature changes in the fallopian canal of cadaveric mastoid bones during facial recess drilling using diamond burs demonstrated that there was a rise in temperature in both the I and NI groups; however, irrigation significantly reduced the temperature changes. The change in temperature of the NI group was significant enough to produce permanent injury to the facial nerve, according to studies on thermal injury to peripheral nervous tissue. On the basis of this study, it can be concluded that constant irrigation should be used during mastoid drilling to minimize thermal changes, a potential source of injury in approaching the facial recess.

ACKNOWLEDGMENT — The authors thank Dr Michael Donnelly for his involvement in the statistical analysis of the data.

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COSMETIC FACIAL SURGERY

The Indian Society of Facial Plastic and Reconstructive Surgery Inaugural Conference and Workshop on Cosmetic Facial Surgery will be held December 7-9, 2001, in Indore, India. For information, please contact Dr Subramaniam Iyyer (subu@md3.vsnl.net.in) or Dr Brajendra Baser, 109 Trade House South Tukoganj Indore MP 452001, India (brajendrab11@hotmail.com, baser@vsnl.com).

MULTICENTRICITY OF INTRAPAROTID FACIAL NERVE SCHWANNOMAS

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Facial nerve schwannomas are uncommon neoplasms. Multiple schwannomas of the facial nerve in the parotid region are rare. Research regarding the pathogenesis of multiple facial nerve schwannomas is incomplete. Both the neoplastic bridging of tumor cells and tumor multicentricity have been hypothesized. We present a case of multiple intraparotid facial nerve schwannomas. In this case, the histologic features of the tumors support the multicentric hypothesis.

KEY WORDS — facial nerve neoplasm, intraparotid neoplasm, multiple facial nerve schwannomas.

INTRODUCTION

Facial nerve schwannomas (neurilemmomas, neurinomas) are uncommon, slow-growing neoplasms that may occur from the cerebellopontine angle to the peripheral branches of the facial nerve. The majority of facial nerve schwannomas are intratemporal, with 9% of cases arising from the intraparotid portion.[1] Multiple schwannomas of the facial nerve are rare, especially in the parotid region. Previous studies described either neoplastic bridging of the tumor cells[1,2] or tumor multicentricity[3,4] as the cause of multiple intraparotid facial nerve schwannomas. This article describes the case of a 27-year-old woman with multiple facial nerve schwannomas in her parotid region and discusses the histologic features of the tumors. Our present data support the hypothesis of tumor multicentricity for the pathogenesis of multiple facial nerve schwannomas.

CASE REPORT

A 27-year-old woman complained of a painless mass in the right preauricular area that had been present for 3 months. Physical examination revealed a 4 × 4-cm, partially mobile and nontender mass between the tragus and the mandibular angle. The facial nerve functioned normally. There was no evidence of von Recklinghausen's disease. A contrast-enhanced computed tomography scan revealed a 3.5 × 3.5-cm multilobulated mass in the right parotid gland (Fig 1).

During the operation, several elastic and yellowish encapsulated tumors were noticed in the parotid gland. The main trunk of the facial nerve was encased by tumors 1 cm distal to the stylomastoid foramen. Failing to dissect the tumor from the nerve, we sacrificed a segment of facial nerve (including the main trunk and part of the zygomaticofacial and cervicofacial divisions) and then repaired it with a cable graft from the greater auricular nerve. The patient awoke from the operation with a House-Brackmann grade V facial weakness. With maximum effort, she could nearly close her right eye, but other movements on the right side of the face remained paralytic.

Gross examination of the specimen revealed 8 separate tumors (Fig 2). The tumor encasing the main

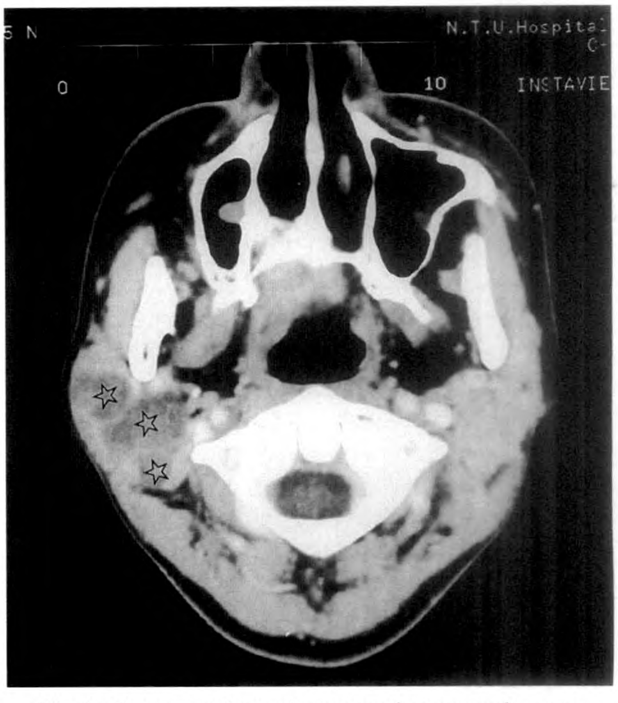

Fig 1. Contrast-enhanced computed tomography scan shows multilobulated mass in right parotid area (stars).

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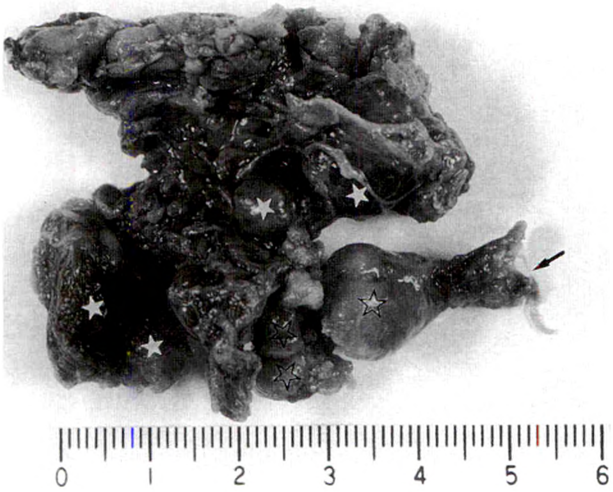

Fig 2. Multiple facial nerve schwannomas. Tumor encasing main trunk of facial nerve (arrow) was dissected for frozen section. Another 3 adjacent tumors (open stars) and 4 separate tumors (white stars) were also disclosed.

trunk of the facial nerve was dissected for frozen section. The cut surface was relatively homogeneous and grayish yellow in color. The second tumor was connected to the first tumor, and another 2 tumors were adjoined to the second. The remaining 4 tumors were separate.

Histopathologically, the schwannomas were uninodular masses surrounded by fibrous capsules consisting of both epineurium and residual nerve fibers. The hallmark of these tumors was a pattern of alternating Antoni A (Fig 3A) and B type areas. Occasionally, a transition between the normal neural tissue and tumor was discovered (Fig 3B). The immunohistochemical stain for neurofilament protein demonstrated residual nerve fibers within these tumors (Fig 3C).

DISCUSSION

Schwannomas are benign tumors that arise from the nerve sheath. The majority of facial nerve schwannomas are intratemporal, with 9% of cases arising from the intraparotid portion.[1] The preoperative diagnosis is difficult because of the infrequency of the disease. The estimated incidence of "parotid" tumors of facial nerve origin ranges from 0.2% to 1.5%.[5] Bretlau et al[6] reviewed 52 cases of intraparotid schwannomas, in which only 4 patients had a correct preoperative diagnosis. The results of fine-needle aspiration cytology are usually inconclusive. They usually reveal myxoid material or occasionally a few bland spindle cells, which is insufficient for cytologic diagnosis.[7]

Facial nerve schwannomas are solitary, encapsulated tumors that are usually attached to a nerve. They appear to push the nerve aside and allow the surgical removal of the tumor with preservation of the nerve. Schwannomas have 2 histologic patterns: a compact spindle-cell pattern with nuclei palisading to form Verocay bodies (Antoni type A), and a looser and a more pleomorphic pattern (Antoni type B).[8] This case presents the characteristic picture of schwannomas that contain both Antoni A and B type patterns. However, the facial nerve passed directly through the tumor, rather than being pushed aside, which is uncommon for schwannomas.

The pathogenesis of multiple facial nerve schwannomas is not clearly understood. Conley and Janecka[3] reviewed 23 Schwann cell tumors of the facial nerve in 17 patients. Three patients had multicentric tumors involving multiple branches of the peripheral facial nerve. Koide et al[4] published the first report of intraparotid multiple facial nerve neurinomas in 1966. In this case, the researchers discovered 5 separate intraparotid tumors, each arising from a main branch of the facial nerve. These findings imply that they were not monoclonal tumors. Conversely, Forton et al[1] reported 2 cases of multiple neurilemmomas and demonstrated that schwannoma cells penetrated the nerve fascicles and pursued the natural course of the facial nerve. Forton et al supposed that such a growth pattern accounted for the typical dumbbell shape of facial schwannomas. The bridging of the lobulated schwannomas with neoplastic cells on the grossly normal nerve was also reported.[2] These findings contradict the multicentric hypothesis.

In this case, we identified 8 facial nerve schwannomas in the parotid gland. Apparently, 1 tumor encased the main trunk of the facial nerve, and there were 3 adjacent tumors. However, the remaining 4 tumors were separate and possessed an indistinct relationship with the facial nerve. These tumors may have arisen from small branches of the facial nerve that were not identified during the operation, ie, in correlation with the multicentric hypothesis, or they may have sprouted from the main part of the tumor and then split into individual tumors during surgical manipulation.

Histologically, the tumors were well circumscribed by fibroareolar tissue. The tumor cells did not penetrate the nerve fascicles, nor did they "bridge" along the nerve. Immunohistochemical studies also failed to display a connection between the separate tumors. Additionally, we recognized an area in which a grossly normal nerve was about to transform into a schwannoma. On the basis of these findings, this study supports the multicentric hypothesis of multiple facial nerve schwannomas.

CONCLUSION

Multicentric schwannomas of the facial nerve in

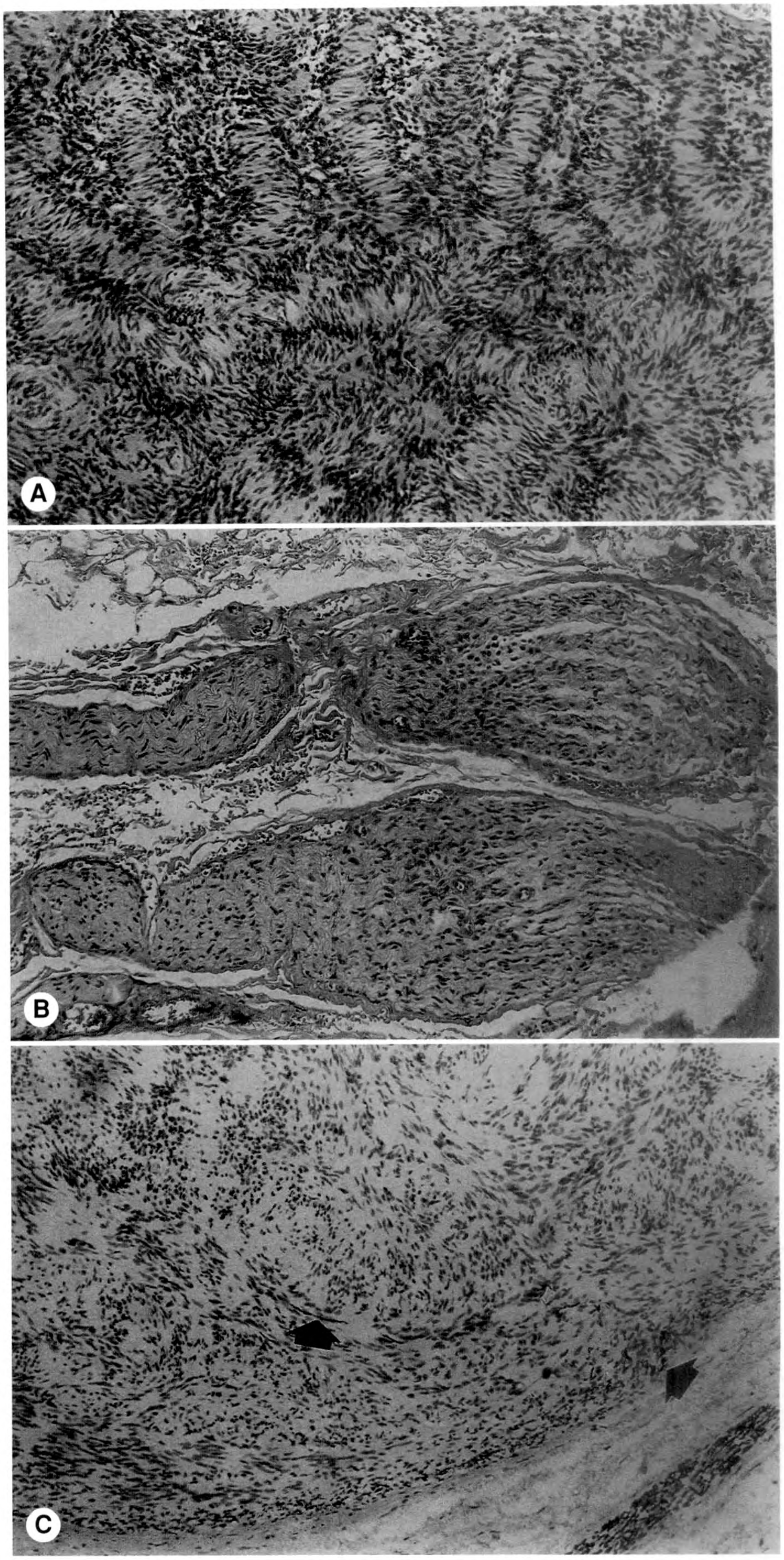

Fig 3. Histopathologic findings (original ×66). **A)** Antoni A area of tumor illustrates prominent nuclear palisading and whorling of cells within schwannoma (H & E). **B)** Transition between normal neural tissue and tumor (H & E). **C)** Immunohistochemical study for neurofilament protein illustrates residual facial nerve fibers within tumor (arrows; avidin-biotin complex method).

the parotid region are exceedingly rare. The exact process by which multiplicity develops is unclear. However, our finding of an impending schwannoma developing from a grossly normal nerve sheath may support the multicentric hypothesis of multiple facial nerve schwannomas.

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Ann Otol Rhinol Laryngol 110:2001

OTOPATHOLOGY IN A CASE OF TYPE I WAARDENBURG'S SYNDROME

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We report a case of type I Waardenburg's syndrome that provides insight into the etiopathogenesis of sensorineural hearing loss (SNHL) in this syndrome. The subject, a 76-year-old woman with type I Waardenburg's syndrome (dystopia canthorum, heterochromia irides, and white hair), had congenital low-frequency SNHL in her right ear only, which had remained relatively stable throughout her life. Blood leukocyte DNA studies revealed a PAX-3 mutation with a 1 base pair C-to-A substitution in exon 5 at base 602. Light microscopic studies of the right cochlea showed intact neurosensory structures in only the lower basal turn, with the remainder of the cochlea showing absence of melanocytes, absence of stria vascularis, missing hair cells, dysmorphogenesis of the tectorial membrane, and lack of peripheral processes of the spiral ganglion cells. There was pathological alteration of the vestibular dark cells with marked reduction of melanocytes associated with these dark cells. The left inner ear was normal, with a full complement of neurosensory structures, including melanocytes. Because the PAX-3 gene is involved in neural crest development and melanocytes migrate from the neural crest to the ear, the findings in this case are consistent with the hypothesis that defective melanocyte migration or defective melanocyte function results in defective development of the stria vascularis (and perhaps other structures of the ear), leading to SNHL.

KEY WORDS — gene mutation, histopathology, temporal bone, Waardenburg's syndrome.

INTRODUCTION

Waardenburg's syndrome (WS) was first described in 1951 as an autosomal dominant disorder characterized by dystopia canthorum (lateral displacement of inner canthi of eyes), pigmentary abnormalities (eg, white forelock, heterochromia irides, premature graying of hair), and sensorineural hearing loss (SNHL).[1] The syndrome was subsequently classified into type I (WS-I; with dystopia canthorum) and type II (WS-II; without dystopia canthorum).[2] The incidence of SNHL in WS-I ranges from 30% to 70%.[3] The hearing loss is highly variable[1,3-5]; it can be unilateral or bilateral, and can range in severity from mild to profound. The hearing loss is congenital and usually nonprogressive. Patients with partial SNHL characteristically show a greater loss in the lower frequencies. WS-I is caused by mutations at the PAX-3 locus on 2q35.[6-8] The PAX-3 gene codes for a transcription factor that regulates expression of other genes by binding to DNA, and is known to be involved in development of the neural crest.[9] In this report, we present the temporal bone histopathologic findings in a patient with WS-I who was known to have a PAX-3 mutation in DNA from peripheral lymphocytes. This case provides interesting insights into the etiopathogenesis of SNHL in WS-I.

CLINICAL HISTORY

The subject of this report belonged to a family with WS-I. She had dystopia canthorum, heterochromia irides, white hair, and hearing loss (WS-I). Genomic DNA was isolated from her peripheral blood lymphocytes by standard methods,[6] which revealed a PAX-3 mutation with a C-to-A substitution at base 602 in exon 5. (Serine was changed to a termination codon.) Her daughter and son are both affected with WS-I, her daughter has congenital profound deafness, whereas her son has near-normal hearing.

In 1965, at age 46, our subject became a temporal bone donor with the (former) National Temporal Bone Banks Program (now the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry). An otorhinolaryngological evaluation at that time revealed a history of congenital hearing loss in her right ear without any other accompanying otologic symptoms. Findings on examination of the ears, nose, and throat were unremarkable. Audiometric testing at age 46 revealed a low-frequency SNHL in the right ear with thresholds of 35 to 40 dB for 250 to 1,000 Hz, normal hearing at other frequencies in the

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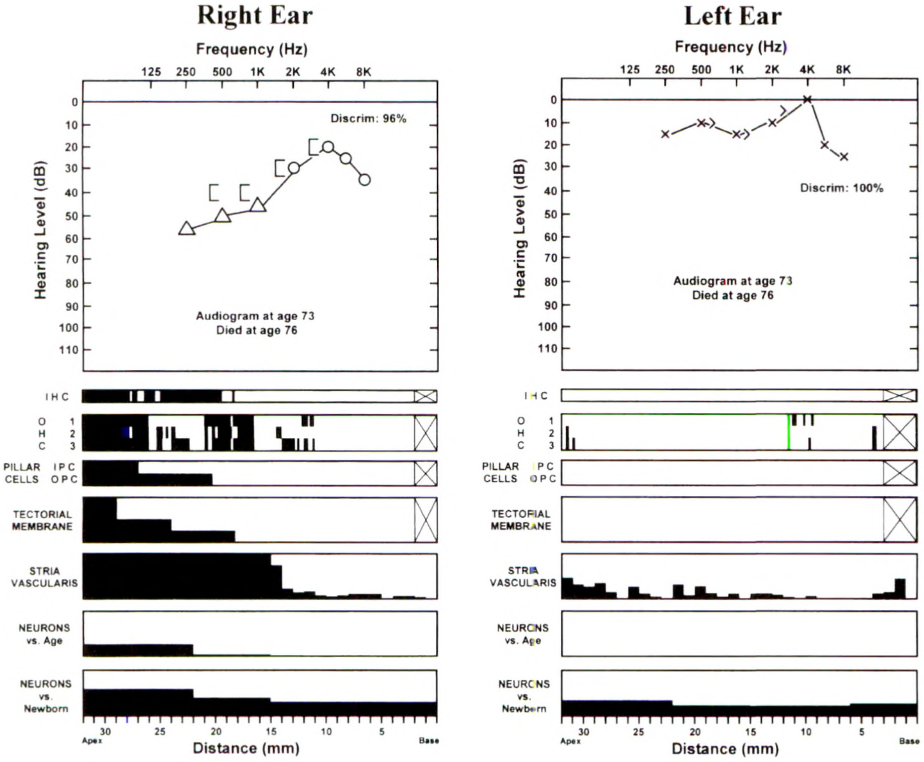

Fig 1. Audio-cytocochleograms. Audiogram was performed at 73 years of age, 3 years before death. Cytocochleograms represent quantitative estimates of sensory and neural structures depicted as histograms, from base to apex of cochlea, according to technique outlined by Schuknecht.[5] Black areas represent missing or abnormal values, and areas marked by X represent areas in cochlea in which cytologic evaluations could not be made. Hair cells and pillar cells were recorded as being present or absent. Tectorial membrane was recorded as being normal or dysmorphic. Atrophy of stria vascularis was estimated as percentage and is depicted on ordinate. Numbers of spiral ganglion cells were counted in 4 segments of cochlea from base to apex, and depicted as percentage of count expected at birth and for age-matched controls. IHC — inner hair cells, OHC — outer hair cells, IPC — inner pillar cells, OPC — outer pillar cells.

right ear, and normal hearing (≤20 dB) at all frequencies in the left ear. Her hearing loss progressed very gradually over the ensuing 3 decades. Audiometric evaluations at ages 57, 69, and 71 revealed a 50- to 60-dB low-frequency SNHL with >90% speech discrimination in the right ear, and thresholds of ≤20 dB in the left ear. Her last audiogram was at age 73 in 1992, 3$^1/_2$ years prior to death, and it revealed a 50- to 55-dB low-frequency SNHL in the right ear with 96% speech discrimination (Fig 1[5]). She died at age 76 of cancer. The body was refrigerated immediately after death, and both temporal bones were harvested at 56 hours postmortem.

TEMPORAL BONE HISTOPATHOLOGY

Both temporal bones were processed in the standard manner for light microscopy, including fixation with 10% neutral buffered formalin, decalcification with ethylenediaminetetraacetic acid, embedding in celloidin, serial sectioning in the axial plane at a thickness of 20 µm, and hematoxylin and eosin staining of every 10th section.[5] The stained histologic sections were examined by light microscopy. Graphic reconstruction of the cochlea was performed according to the method described by Schuknecht[5] to determine loss of various neurosensory elements such as hair cells, pillar cells, stria vascularis, tectorial membrane, and spiral ganglion cells. Spiral ganglion cell counts were compared to normative data reported by Schuknecht.[5]

Right Ear. The temporal bone specimen was complete and included the external auditory canal, middle ear, mastoid air cells, and inner ear. Postmortem preservation of cytologic elements within the inner ear was judged to be good. The external auditory canal, tympanic membrane, middle ear space, ossicles, eustachian tube, mastoid air cells, and petrous apex were normal. The otic capsule was normal.

The bony cochlea was fully developed, with 2$^1/_2$ turns. The cochlear duct appeared normal only in the lower part of the basal turn (Figs 1-3), in which there was a well-developed organ of Corti with both outer and inner hair cells, a healthy-appearing stria vascularis with melanocytes, a normal-appearing spiral ligament, nerve fibers within the osseous spiral lamina, and melanocytes in the modiolus and adjoining areas of the scalae. The cochlear duct and its neurosensory structures were abnormal in the remaining turns of the cochlea (Figs 1-3): both outer and inner hair cells were missing, the stria vascularis was absent in most areas and was dysplastic in some, and the tectorial membrane had eosinophilic material adjacent to and attached to it in the apical turn. There were no melanocytes within the modiolus and adjoining areas of the scala vestibuli and scala tympani (in which they should be normally present; Fig 3) in the middle and apical turns. The spiral ganglion cell count (21,915) was mildly reduced, as compared to age-matched controls (Fig 1).[5] The peripheral processes of the ganglion cells were missing to a severe degree in both the middle and apical turns (Fig 2). The spiral ligament showed atrophy in the middle and api-

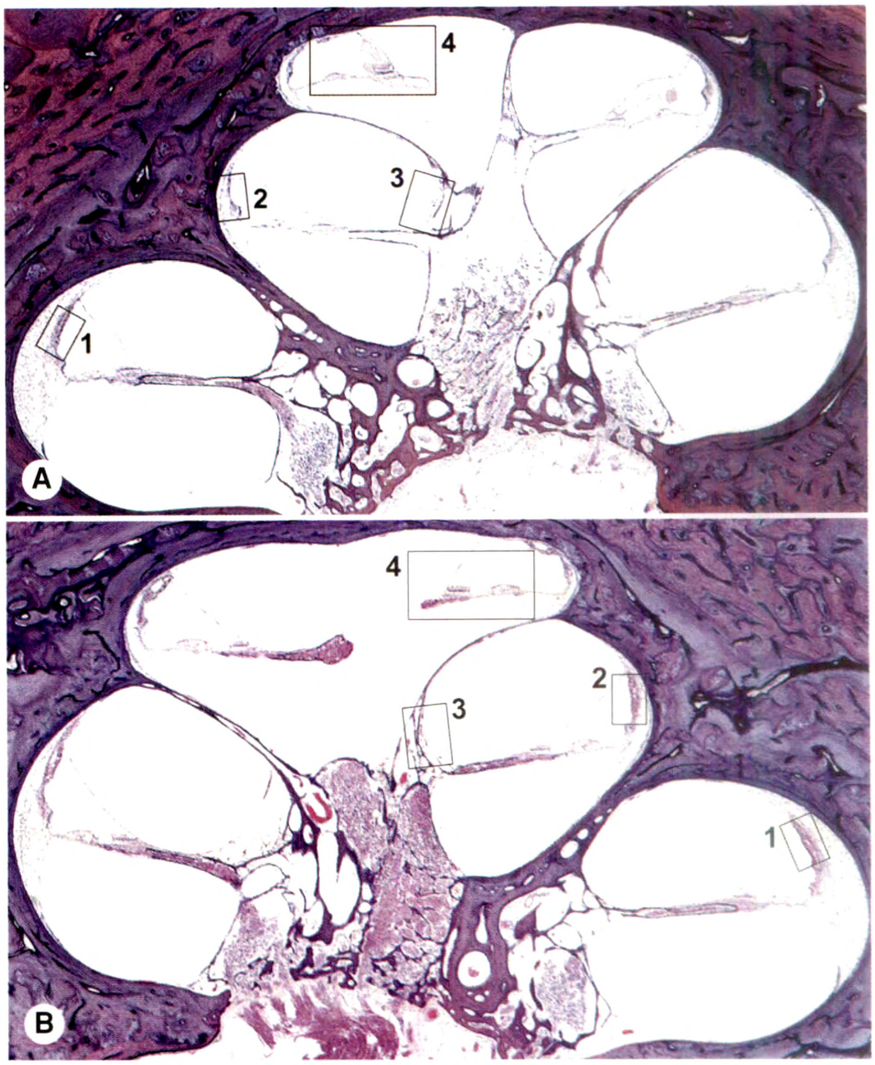

Fig 2. Low-power views of cochleae (original ×24). Boxed areas numbered from 1 to 4 are shown at higher power in Fig 3. **A)** Near-midmodiolar section of right ear. Sensory and neural structures are intact in basal turn only. Middle and apical turns show absence of stria vascularis including melanocytes, diffuse loss of outer and inner hair cells, dysmorphogenesis of parts of tectorial membrane, and severe loss of peripheral dendrites of spiral ganglion cells. **B)** Midmodiolar section of left ear. Cochlear, neural, and sensory structures are present in all turns.

cal turns, with greater atrophy in the apical turn. Reissner's membrane showed apical endolymphatic hydrops. Reissner's membrane showed redundancy in many sections in the other cochlear turns (eg, Fig 2), and it is unclear whether this redundancy represents mild endolymphatic hydrops or a developmentally long membrane without hydrops.

There was pathological collapse of the saccular membranous wall onto its macula. Most hair cells were missing from the saccular macula, but supporting cells were still present. The utricle showed pathological collapse of its membranous wall, with encapsulation of portions of the otolithic membrane and dysmorphogenesis of the neuroepithelium with cyst formation. Hair cells were missing to a severe degree within the utricular macula, and nerve fibers were much reduced in its stroma. The ampullae of all 3 semicircular canals showed distortion of the membranous walls and pathological collapse with scattered ruptures. The perilymphatic trabecular meshwork was disrupted and missing in many areas. Hair cells were missing to a severe degree in all 3 cristae (Fig 4). Dark cells of the ampullae and utricle appeared abnormal, with pyknosis of the nuclei, cyst formation, and areas in which the dark cells were distributed in clumps. Melanocytes associated with the dark cells were absent in most sections (Fig 4) and markedly reduced in others. Melanocytes were not seen in the subepithelial regions of the intraosseous endolymphatic sac. The remainder of the endolymphatic duct and sac and the vestibular aqueduct were unremarkable. Blood vessels were present within the modiolus, lateral cochlear wall, and vestibular sense organs in the expected locations, but a quantitative assessment of the vascular supply to the inner ear was not done. The cochlear aqueduct was patent and appeared normal. There was avulsion of the cochlear, vestibular, and facial nerves from the internal auditory canal. The labyrinthine, geniculate, tympanic, and mastoid segments of the facial nerve appeared normal.

Left Ear. The specimen contained the external ear, middle ear, and inner ear, as well as a portion of the trigeminal ganglion. Postmortem preservation of cy-

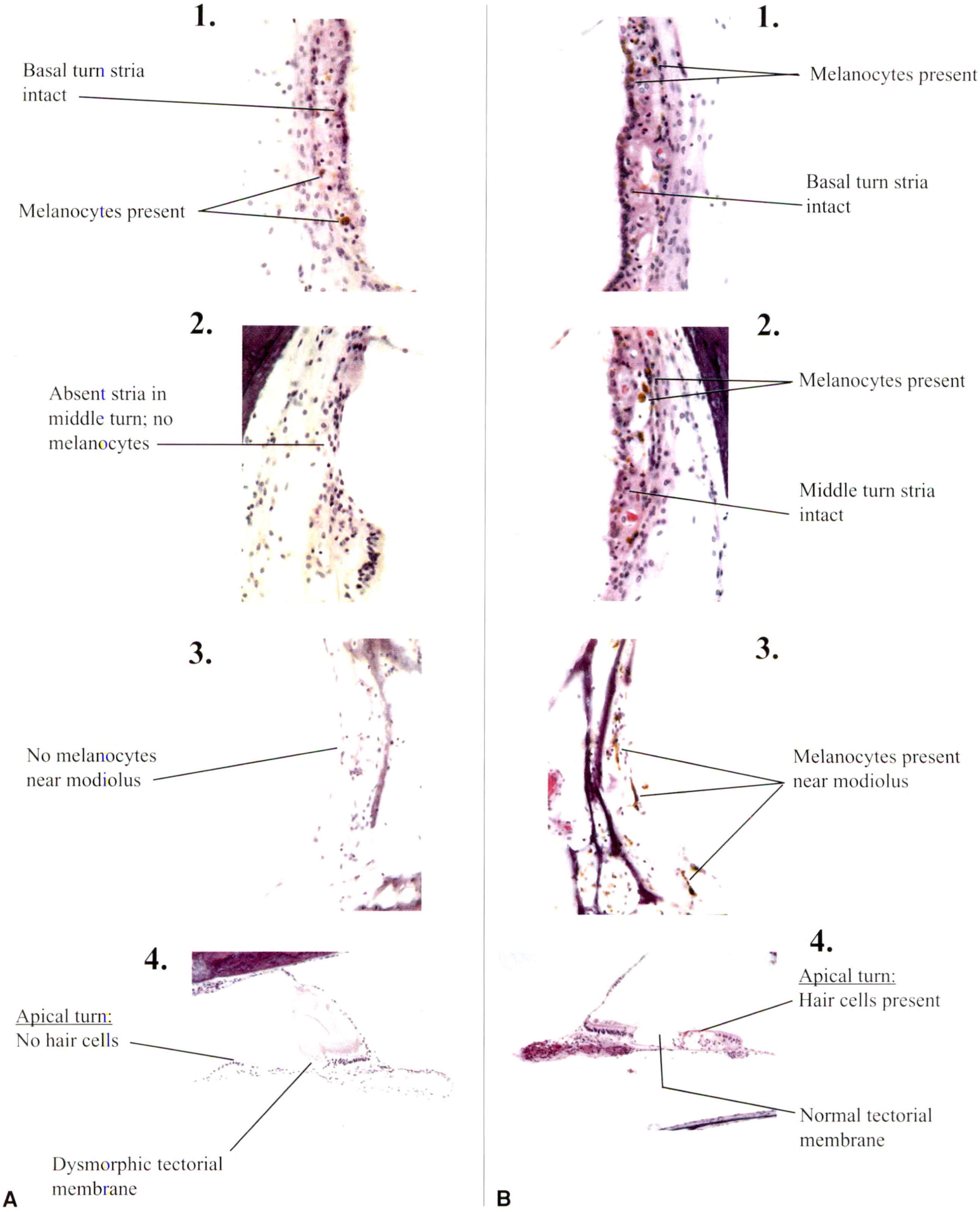

Fig 3. Higher-power views of selected areas (numbers 1 through 4) of cochleae from Fig 2. **A)** Right ear. 1 — Stria vascularis from basal turn shows that all 3 strial layers are intact and melanocytes are present (original ×165); 2 — middle turn shows absent stria with no melanocytes (original ×165); 3 — scala vestibuli region near modiolus shows absence of melanocytes (original ×125); 4 — apical turn shows lack of hair cells in organ of Corti (original ×64). Tectorial membrane is dysmorphic, with eosinophilic material attached to it and near it. **B)** Left ear. 1 — Basal turn shows intact stria vascularis with melanocytes (original ×165); 2 — middle turn also shows intact stria with melanocytes (original ×165); 3 — scala vestibuli near modiolus shows that melanocytes are present (original ×125); 4 — apical turn shows organ of Corti with outer and inner hair cells and normal tectorial membrane (original ×64).

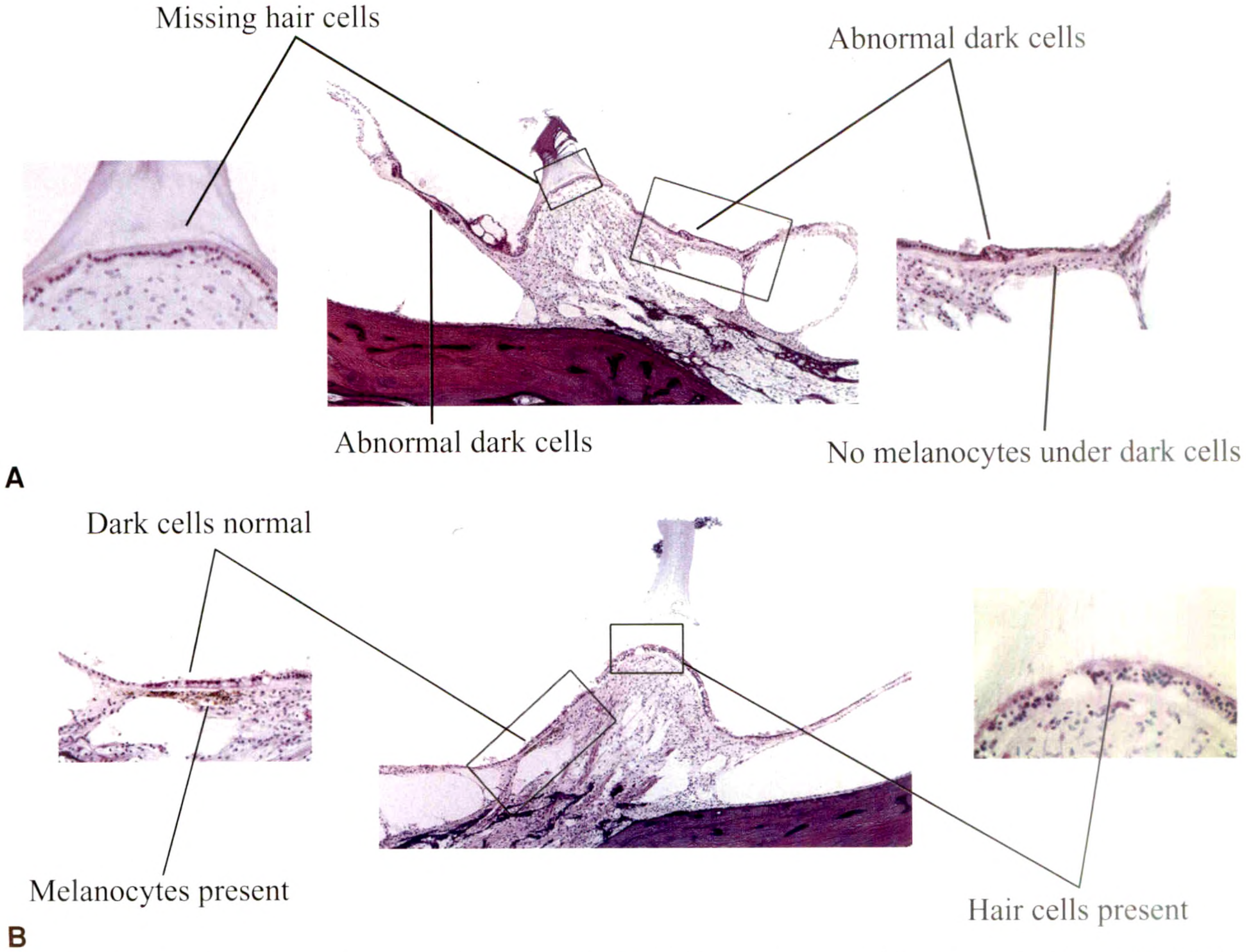

Fig 4. Ampullae of lateral semicircular canals (original ×35). Boxed areas are shown at higher power (original ×70 for dark cells and original ×140 for neuroepithelium). **A)** Right ear. Neuroepithelium is missing hair cells. Dark cells appear abnormal, with areas of cyst formation. There are no melanocytes under dark cells. **B)** Left ear. Neuroepithelium appears normal, with intact hair cells and supporting cells. Dark cells appear normal, including associated melanocytes.

tologic elements was judged to be good. The external auditory canal, tympanic membrane, middle ear space, ossicles, mastoid air cells, eustachian tube, and petrous apex appeared normal. The otic capsule was normal. The cochlea was normally developed, with $2^1/2$ turns. Reissner's membrane showed apical endolymphatic hydrops. Inner hair cells were present throughout the cochlea. Outer hair cells were also present in all turns, with the exception of minor scattered losses in the basal and apical turns (Fig 1). The stria vascularis appeared to be normally developed in all turns, including melanocytes. There was mild, patchy atrophy of the stria, especially in the more apical regions of the cochlea. Spiral ganglion cells were present in good numbers (total count, 26,613) and were judged to be normal for the subject's age. The spiral ligament showed atrophy in the more apical turns that was also thought to be consistent with age-related changes. Large numbers of melanocytes were present within the modiolus and adjoining regions of the scala vestibuli and scala tympani in all turns (Fig 3). The saccule, utricle, cristae, and semicircular ducts were normal, including the hair cells, supporting cells, dark cells, melanocytes, and membranous walls (Fig 4). Scarpa's ganglion showed a good population of cells. The endolymphatic sac (including melanocytes in the subepithelial region of the intraosseous sac) and vestibular aqueduct were normal. The cochlear aqueduct was patent. The facial nerve was normal in its course through the temporal bone.

DISCUSSION

Melanocytes are widely distributed within the cochlear and vestibular sense organs: in the stria vascularis (where they constitute its intermediate cell layer), in the modiolus and adjoining regions of the scala vestibuli and scala tympani, near the dark cells of the ampullae and utricle, and in the subepithelial tissue of the intraosseous endolymphatic sac.[10-13] The melanocytes contained within the inner ear are believed to have migrated from the neural crest.[5] Although the precise role of melanocytes in auditory and vestibular function remains unknown,[5,10-13] it is established that SNHL is frequently observed in pigmentary disorders.[1,5,14,15]

Schrott and Spoendlin[15] investigated the pathogenesis of SNHL associated with pigment anomalies such as those that accompany WS by studying the W/W^v mouse (so-called black-eyed white mutant mouse). They found a failure of the melanocytes to migrate from the neural crest to the inner ear that resulted in lack of development of the stria vascularis. They also found that degeneration of hair cells, the organ of Corti, and spiral ganglion cells occurred postnatally, beginning at about 1 month of age. They concluded that SNHL in WS is presumably the result of a lack of strial development, and that the other alterations were secondary. Steel et al[16-18] also investigated the role of melanocytes in the stria vascularis by studying its development in the W^v/W^v mouse mutant (viable dominant spotting mouse), which is known to have a primary neural crest defect leading to absence of melanocytes in the skin. The investigators used light and electron microscopy, as well as functional measurements of the endocochlear potential, and concluded that melanocytes had an important role in stria vascularis development and in production of the endocochlear potential. Although their studies clearly indicated that the primary cause of strial dysfunction was an absence of melanocytes, it is not yet clear what aspect of melanocyte function is important for their role in the stria. Melanocyte function in the stria is apparently not dependent upon their ability to produce melanin, because there is no evidence that the striae of albino animals that contain amelanotic melanocytes are dysfunctional.[16]

There are few human temporal bone case reports of otopathology in WS. Fisch[19] reported on the temporal bones of a 3-year-old girl with profound SNHL and WS-I. There was marked atrophy of the stria vascularis, absence of hair cells throughout the cochlea, and a severe decrease in the spiral ganglion cell population, but preservation of the vestibular sensory cells. No comment was made about the distribution of melanocytes within the inner ear. Rarey and Davis[20] described otopathological findings in a 2-year-old boy with WS-II who also had Hirschsprung's disease. There was diffuse atrophy of the organ of Corti, stria vascularis, and vestibular sense organs and nerves. Nakashima et al[21] reported the temporal bone findings in a 3-year-old girl with WS-II and Hirschsprung's disease who was also profoundly deaf in both ears. There was severe cochleosaccular atrophy and absence of melanin pigment within the inner ear. These previous temporal bone case reports on patients with WS have severe atrophy of the organ of Corti and stria vascularis as a common finding. However, the severe degree of otopathological changes and profound hearing loss in these case reports does not permit one to draw any definitive conclusion about the association between melanocytes and cochlear disease. The present case is unique in that our subject had partial and asymmetric SNHL, thus permitting correlation between the presence or absence of melanocytes and cochlear disease, and also unique in that the precise genetic mutation had been positively identified.

The PAX-3 gene codes for a transcription factor that regulates expression of other genes by binding to DNA, and is known to be involved in development of the neural crest.[6-9] In the developing embryo, melanocytes migrate from the neural crest to the otic vesicle, in which they become localized below the target epithelia, ie, the areas that will develop into the stria vascularis and the vestibular dark cells.[22] Development and migration of the melanocytes proceeds from the base to the apex of the cochlea, and separately (but simultaneously) in the vestibular system. In our subject's right ear (which had good hearing only in the high frequencies), there was a striking association between the presence of melanocytes and a lack of cochlear abnormalities. Melanocytes were present in the basal turn, wherein the stria and other structures of the cochlear duct were well developed and appeared to be normal (Figs 2 and 3). In the middle and apical turns, there were an absence of the stria, including melanocytes, an absence of melanocytes in and near the modiolus, a diffuse loss of inner and outer hair cells, dysmorphogenesis of the tectorial membrane, and an absence of peripheral dendrites of the spiral ganglion cells (Figs 2 and 3). In the contralateral left ear with good hearing, the neurosensory structures of the cochlear duct, including melanocytes, appeared to be normally developed (Figs 2 and 3). Thus, our findings provide support for the hypothesis that SNHL in WS-I results from abnormal migration and/or function of neural crest–derived melanocytes, leading to defective development of the stria vascularis and perhaps other structures of the cochlea. This hypothesis can explain the phenotypic variability seen in WS-I: the SNHL may be bilateral, unilateral, or nonexistent, depending on whether some of the melanocytes escape the effects of the mutation. Because melanocytes migrate from the base to the apex within the developing cochlea, the above hypothesis can also explain the characteristic low-frequency SNHL that is often observed in WS-I, as in our subject. The molecular mechanism that permits some of the melanocytes to escape the effects of the mutation remains unclear. It does not appear to be the result of allelic heterogeneity.[23,24] Genetic background, modifier genes, stochastic events occurring during embryogenesis, and nonrandom environmental factors have all been proposed as being responsible for the observed phenotypic variability.[23,24]

The correlation between melanocytes and pathological findings is also evident within the vestibular system. In the right ear, there were markedly reduced numbers of melanocytes associated with dark cells (Fig 4). The dark cells showed pathological changes, including pyknosis of the nuclei, cyst formation, and areas in which they were distributed in clumps. There was also pathological collapse of the membranous walls of the saccule, the utricle, and all 3 ampullae. Most hair cells were missing from the maculae and cristae, and there was dysmorphogenesis of both the utricular and saccular neuroepithelia. The vestibular system and melanocytes in the contralateral left ear were normal (Fig 4). In both the cochlear and vestibular systems, it cannot be determined whether the loss of sensory hair cells and other abnormalities (affecting the tectorial membrane, peripheral dendrites, membranous walls of the labyrinth, etc) were primarily due to melanocyte dysfunction, or whether they were secondary to absence of the stria and dark cells. Even though the patient's left ear had near-normal hearing, the hearing threshold at 4 kHz was 10 to 15 dB better than the thresholds for the lower frequencies (Fig 1). This difference may represent normal variation, or it could represent a subtle degree of cochlear dysfunction in the more apical regions that existed as part of the WS-I syndrome, although at a level undetectable by light microscopy.

The temporal bone changes in the right ear in this case also demonstrate the insensitivity of the pure tone audiogram to variations in cochlear damage in cases of low-frequency SNHL.[5] Despite complete absences of hair cells, stria vascularis, and peripheral processes of spiral ganglion cells in the middle and apical turns, the audiogram only showed a 50- to 60-dB low-frequency loss. The low-frequency thresholds possibly reflected a longitudinal spread of the field of excitation of the basilar membrane toward the more basal, functioning areas of the cochlea.[5,25-27] The findings have implications regarding hearing aid fitting in ears with low-frequency SNHL: other things being equal, ears with nonfunctional apices (such as the present case) will derive little or no benefit from amplification, in contrast to ears with functioning inner hair cells and neurons in the apex. A number of psychophysical tests can be used in the clinical setting to supplement the audiogram for proper diagnosis of the status of the cochlear apex and thereby aid in recommendations for amplification.[25-27]

ACKNOWLEDGMENTS — The authors are indebted to the subject and her family members, not only for donating their tissues, but also for a high level of enthusiasm and interest in this research effort. We are also grateful to Robert Goldstein, PhD, for providing some of the audiological data, and to Richard Cortese for the photomicrographs. The NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry coordinated the pledging and retrieval of the temporal bones.

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Ann Otol Rhinol Laryngol 110:2001

HISTOPATHOLOGY OF COCHLEAR IMPLANTS IN HUMANS

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The insertion of an intrascalar electrode array during cochlear implantation causes immediate damage to the inner ear and may result in delayed onset of additional damage that may interfere with neuronal stimulation. To date, there have been reports on fewer than 50 temporal bone specimens from patients who had undergone implantation during life. The majority of these were single-channel implants, whereas the majority of implants inserted today are multichannel systems. This report presents the histopathologic findings in temporal bones from 8 individuals who in life had undergone multichannel cochlear implantation, with particular attention to the type and location of trauma and to long-term changes within the cochlea. The effect of these changes on spiral ganglion cell counts and the correlation between speech comprehension and spiral ganglion cell counts were calculated. In 4 of the 8 cases, the opposite, unimplanted ear was available for comparison. In 3 of the 4 cases, there was no significant difference between the spiral ganglion cell counts on the implanted and unimplanted sides. In addition, in this series of 8 cases, there was an apparent negative correlation between residual spiral ganglion cell count and hearing performance during life as measured by single-syllable word recognition. This finding suggests that abnormalities in the central auditory pathways are at least as important as spiral ganglion cell loss in limiting the performance of implant users.

KEY WORDS — cochlear implantation, electrode trauma, neo-osteogenesis, spiral ganglion.

INTRODUCTION

The insertion of an intrascalar electrode array during cochlear implantation causes immediate damage to the inner ear and over time may cause additional changes that can interfere with neuronal stimulation. The effects of cochlear implantation have been studied histopathologically on 3 classes of temporal bones: 1) animal bones,[1-10] 2) human bones implanted after death ("cadaveric implants"),[11-14] and 3) human bones from patients who in life had undergone cochlear implantation.[15-24]

Animal models of cochlear implantation offer advantages such as control over individual variability, cause of deafness, and surgical technique, but extrapolation of effects to humans requires assuming similar cross-species responses to trauma, which may not always be valid. Study of normal cadaveric human specimens offers the advantage of differentiating insertional trauma from preexisting damage, but long-term changes, particularly degenerative phenomena among remaining spiral ganglion cells, cannot be studied in this manner. Thus, studies of temporal bones from humans who in life had undergone implantation are essential to better understanding of long-term effects of implantation in humans. To date, however, there have been reports on fewer than 50 temporal bone specimens from patients who had undergone implantation. Furthermore, the majority of these implants were single-channel, whereas the majority of implants inserted today are multichannel systems with longer and possibly more traumatic electrode arrays.

This report presents the histopathologic findings in temporal bones from 8 individuals who in life had undergone multichannel cochlear implantation, with particular attention to 1) the type and location of trauma at the cochleostomy site and to the spiral ligament, osseous spiral lamina, and basilar membrane, 2) delayed changes at the cochleostomy site and along the course of the electrode array, 3) the effect of these

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TABLE 1. PATIENT DATA

Case No.	*Sex*	*Age at Death (y)*	*Age Deaf (y)*	*Age Implanted (y)*	*Years Implanted*	*Implant Type (Ear)*	*Cause of Deafness*	*Preoperative Radiology*	*Operative Findings*
1	F	54	48	52	2	Ineraid (R)	Unknown	CT: diffuse intracochlear opacity, both ears	Bony obliteration of round window and basal turn
2	M	67	65	65	2	Nucleus 22 (R)	Head trauma	Not available	Full insertion without difficulty
3	M	67	47	53	6	House single-channel (L)	Bilateral temporal bone fracture	Polytomography: bilateral temporal bone fractures, no intracochlear bone	Full insertion without difficulty
				59	8	Nucleus 22 (L)			
4	F	70	68	69	1	Ineraid (L)	Streptomycin ototoxicity	MRI: normal	Bony obliteration of round window and basal turn
5	M	84	77	77	7	Nucleus 22 (R)	Unknown	CT: normal	Full insertion without difficulty
6	M	73	65	70	3	Nucleus 22 (L)	Bacterial meningitis	CT: normal	Full insertion without difficulty
				68	5	Single-channel (R)			Fibrosis in basal turn
7	M	70	56	68	2	Ineraid (L)	Pneumococcal meningitis	CT: fibrous occlusion of basal turn	Round window normal; full insertion without difficulty
8	M	72	20	65	5	Ineraid (R)	Bacterial meningitis	CT: normal	Full insertion without difficulty

CT — computed tomography, MRI — magnetic resonance imaging.

changes on spiral ganglion cell counts, and 4) the correlation between performance as measured by speech comprehension and spiral ganglion cell counts and new bone formation within the cochlea.

MATERIALS AND METHODS

The temporal bones were removed, fixed in 10% buffered formalin, and decalcified in ethylenediaminetetraacetic acid. Those specimens in which the electrode array was left in situ were postfixed in 2% osmium tetroxide. All specimens were then dehydrated in graded alcohols. The specimens in which the electrode array was left in situ were exchanged with propylene oxide and embedded in araldite. In

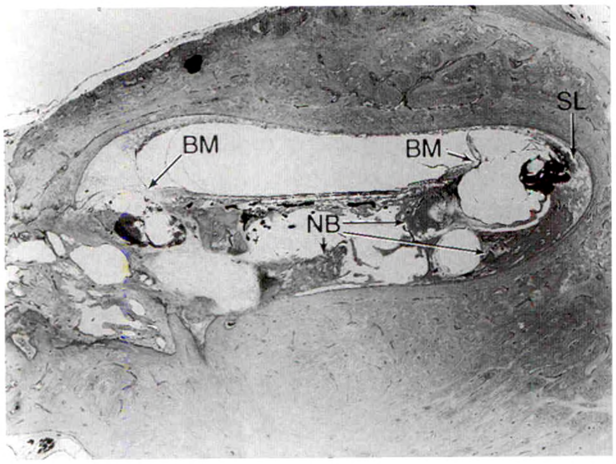

Fig 1. Basal turn near cochleostomy site of case 7, in which there was no radiographic or clinical evidence of new bone formation before operation (original ×18.5). Scala tympani contains new bone (NB). There is trauma to basilar membrane (BM) and to spiral ligament (SL), caused by electrode array.

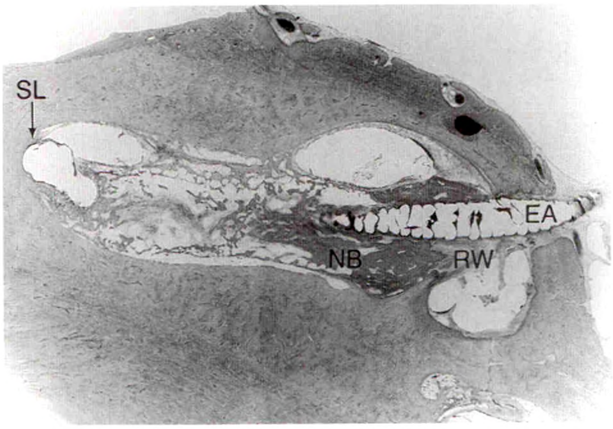

Fig 2. Basal turn near cochleostomy site of case 8, in which there was no radiographic or clinical evidence of new bone formation before operation (original ×15). Electrode array (EA) can be seen entering cochlea near round window (RW). There is new bone (NB) in scala tympani. Basilar membrane of basal turn is displaced, and trauma to spiral ligament (SL) is apparent.

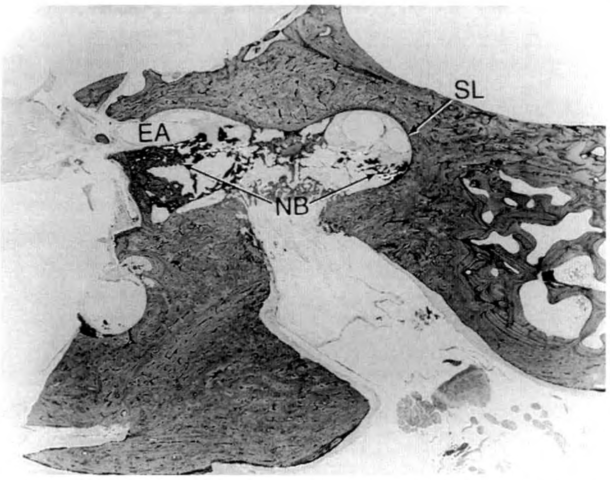

Fig 3. Basal turn near cochleostomy site of case 3, in which there was no clinical or radiographic evidence of new bone formation before operation (original ×9). Electrode array (EA) can be seen entering scala vestibuli, and there is new bone formation (NB) in scala tympani and scala vestibuli. In addition, there is trauma to spiral ligament (SL).

those specimens in which the electrode array was removed before fixation, the temporal bones were embedded in celloidin.

The embedded specimens were serially sectioned in the horizontal (axial) plane at an average thickness of 25 µm. Those specimens embedded in araldite with the electrode array left in situ were sectioned by a technique previously described.[23] For specimens embedded in celloidin, every 10th section was stained with hematoxylin and eosin and mounted on a glass slide. Every 10th section of those specimens embedded in araldite was either left unstained or stained in toluidine blue O before mounting on a glass slide. The serial sections were reconstructed by conventional 2-dimensional methods, including counting of neurons of the 4 segments of the spiral ganglion.[25,26]

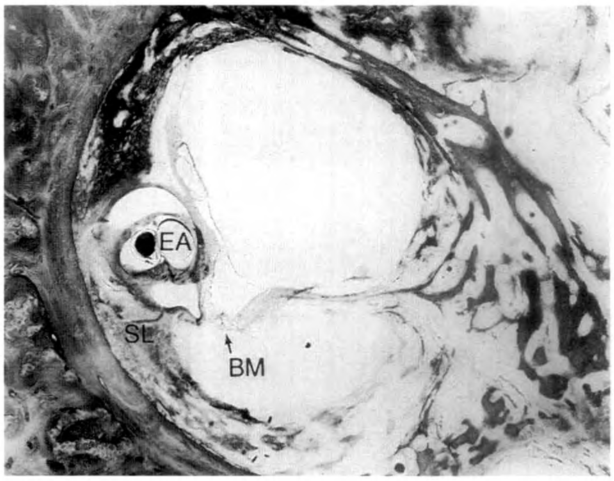

Fig 5. Basal turn of case 7, in which there was no evidence of new bone formation before operation (original ×52). Electrode array (EA) has caused displacement of basilar membrane (BM) and has dissected stria vascularis and spiral ligament (SL).

Correlations were then performed between various histologic findings and the premortem clinical data, including performance with the implant as measured by speech comprehension tests. For uniformity, the NU-6 score without lipreading was used. For the patients for whom a measured NU-6 score was not available (cases 1 and 8), an estimate of the NU-6 score was calculated from available speech reception test scores and their relationship to single-syllable word recognition as described by Rabinowitz et al.[27]

RESULTS

The clinical data on the 8 patients are presented in Table 1. The patients ranged in age from 54 to 84 years and had been implanted from 1 to 8 years before death. In 1 case (case 3), the left cochlea had undergone implantation with a single-channel (House)

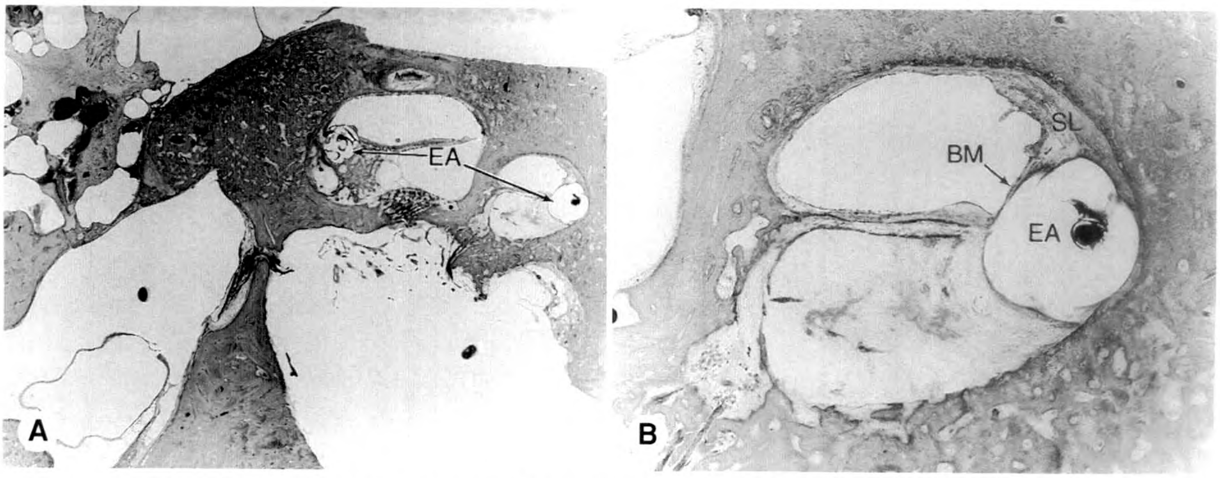

Fig 4. Basal turn of case 4, in which there was evidence of preoperative new bone formation. Electrode array (EA) has displaced basilar membrane (BM) and dissected spiral ligament (SL). **A**) Original ×13.5. **B**) Original ×53.

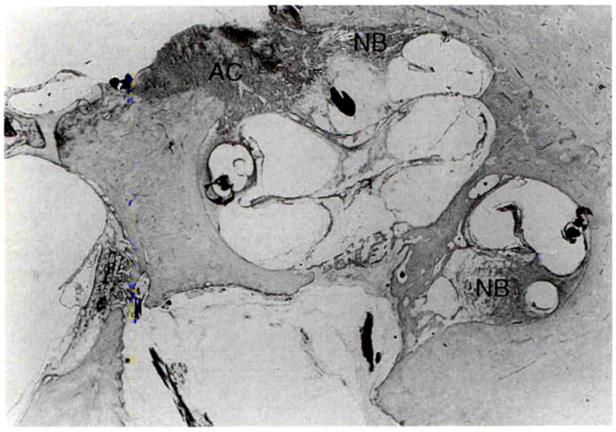

Fig 6. Midmodiolar section of cochlea of case 7, in which apical cochleostomy (AC) had been done (original ×17). There was no evidence of new bone formation before operation. However, there is new bone formation (NB) both in basal turn and at apical cochleostomy.

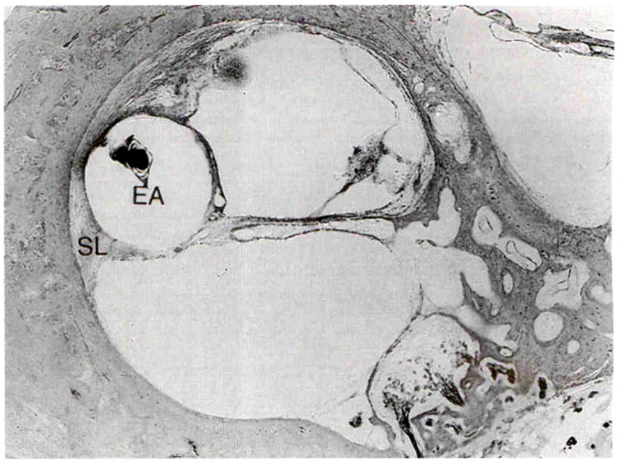

Fig 7. Basal turn of case 8 (original ×45). Electrode array (EA) is in scala vestibuli and has damaged spiral ligament (SL) and is ensheathed in fibrous capsule.

device 14 years before death, followed by explantation and reimplantation of the same cochlea with a Nucleus 22-channel system 8 years before death. In 1 case (case 6), a bilateral cochlear implantation had been done with the Nucleus 22-channel system on the left and a single-channel system on the right. Of the 8 temporal bones from patients who had undergone multichannel implantation before death, 4 had been implanted with the Symbion (Ineraid) device and 4 with the Nucleus 22 device. In 2 cases (cases 1 and 7), an apical cochleostomy[28] was done in addition to the cochleostomy done near the round window. The deafness was attributed to streptomycin ototoxicity in 1, head trauma in 2, and bacterial meningitis in 3, and the cause of deafness was unknown in 2 individuals. Radiographic and clinical intraoperative observations were available for all patients. Clinical and/or radiographic evidence of preoperative fibrosis or new bone formation within the inner ear was present in 3 patients, while in the remaining 5, there was no evidence, either radiographically or clinically, of significant preoperative intracochlear fibrosis or bone formation.

Immediate Trauma Secondary to Implantation. In addition to the trauma induced at the cochleostomy site (Figs 1-3), evidence for immediate trauma to the spiral ligament and stria vascularis was universal, particularly in the ascending limb of the basal turn (Figs 1-5). In all cases, the electrode array appeared to have penetrated the spiral ligament and then to have been deflected by the lateral cochlear wall. This penetration resulted in variable trauma to the spiral ligament and stria vascularis. Distortion or fracture of the osseous spiral lamina occurred in some cases. In 3 cases of implantation of multichannel electrodes,[6-8] the electrode array traversed the scalae, in 1 case from the scala vestibuli to the scala tympani, in another case from the scala tympani to the scala vestibuli, and in the third case from the scala tympani to the scala vestibuli and then back again to the scala

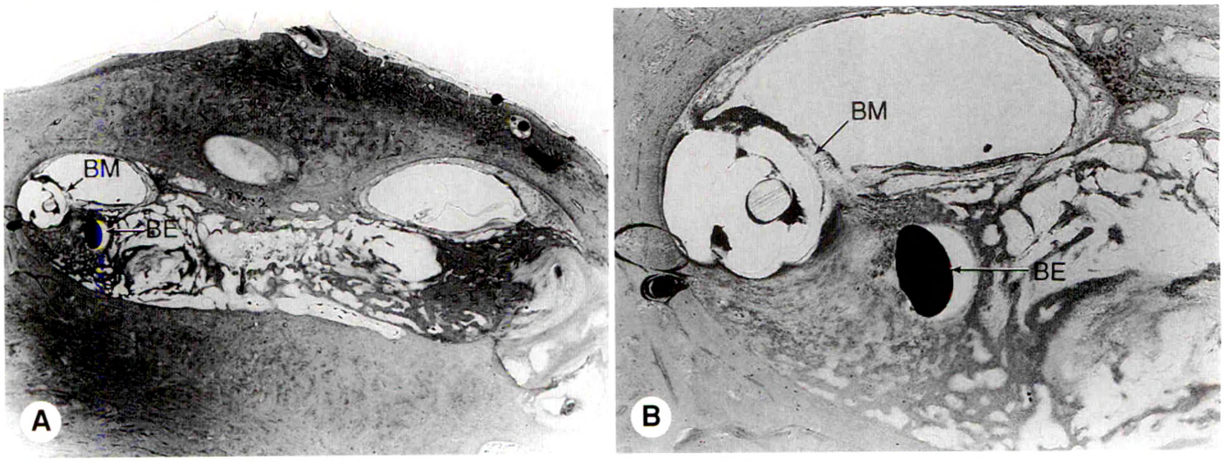

Fig 8. Basal turn of case 8, in which there was no evidence of new bone formation before operation. **A)** Electrode array can be seen elevating basilar membrane (BM; original ×16). Ball electrode (BE) of this Ineraid device is totally embedded in new bone, which is shown in higher magnification in **B** (original ×52).

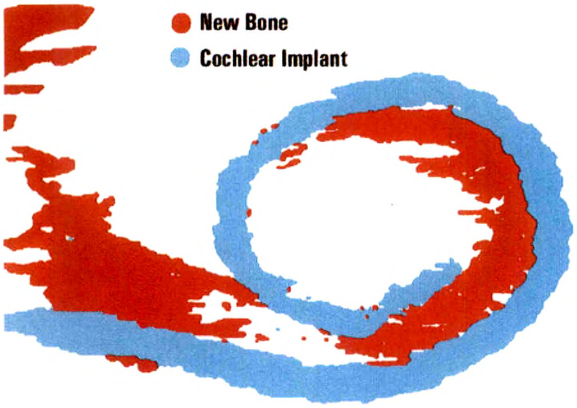

Fig 9. Computer reconstruction of electrode array and new bone formation in case 3, in which there was no evidence of new bone formation within cochlea before operation. There was new bone formation at cochleostomy site and at ascending limb of basal turn.

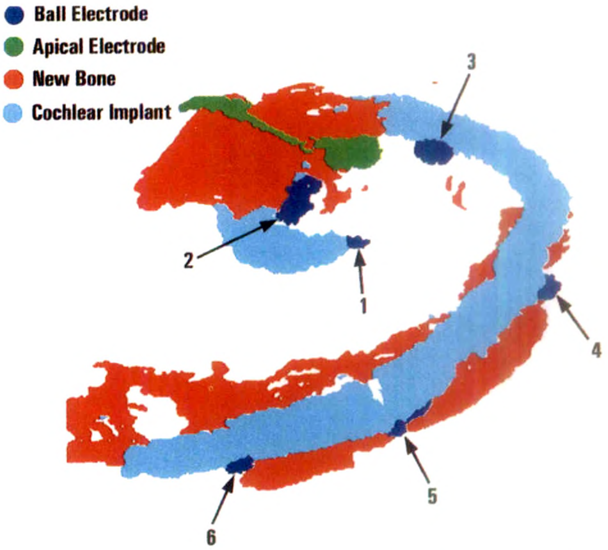

Fig 10. Computer reconstruction of cochlea in case 7, in which there was no evidence of new bone formation before operation. New bone could be seen at cochleostomy site near round window, at apical cochleostomy site, and in ascending limb of basal turn. Numbers 1 through 6 refer to 6 ball electrodes.

tympani. In the other 5 cases, the electrode array stayed within the same perilymphatic scala in which it was originally inserted in the basal turn.

Long-term Changes Induced by Implantation. In all 8 cases, new bone formation occurred at the cochleostomy site in the basal turn (Figs 1-3). In 2 cases (cases 1 and 7), a more apical cochleostomy was performed in addition to that at the round window. New bone formation also occurred at both of these apical cochleostomies (Fig 6). Along the electrode tract, a fibrous sheath surrounding the electrode array was common (Fig 7).

Postimplantation Neo-osteogenesis. In 6 of the cochleas that had been implanted with a multichannel system, there was no radiographic clinical evidence of new bone formation before implantation (Table 1). However, in all 6 patients, some degree of labyrinthitis ossificans was observed histologically. In at least some cases, there was convincing histologic evidence that this new bone formation occurred after implantation. For example, in Fig 8, the electrode array, including a protruding ball electrode, is seen to be totally ensheathed in new bone (ie, ensheathed after implantation was performed). The most common site of postoperative new bone formation, other than the cochleostomy site, was the ascending limb of the basal turn, coinciding with the universal location of trauma to the lateral cochlear wall (Figs 6 and 8-10). The second most common location of new bone formation was the descending limb of the basal turn. In 2 of the 6 cases (cases 2 and 7) with multichannel implants, new bone formation extended into the apical turn, and in only 1 of these (case 7) was an apical cochleostomy performed.

Effects of Implantation and Trauma of Insertion on Spiral Ganglion Cell Counts. In 4 of the 8 cases (cases 2, 3, 5, and 8), the temporal bone of the opposite, unimplanted ear was available for comparison. As shown in Table 2, in 3 of these 4 cases the spiral ganglion cell count in the most basal 2 of 4 segments and the total spiral ganglion cell counts were similar on both sides, and in 2 cases (cases 2 and 8) the spiral ganglion cell counts were greater on the implanted side than on the nonimplanted side. In 3 cases (cases 2, 3, and 8), preoperative audiometry demonstrated a symmetric bilateral profound sensorineural loss. In the third case (case 5), the postimplantation spiral ganglion cell counts were similar on the implanted

TABLE 2. SPIRAL GANGLION CELL COUNTS IN IMPLANTED AND UNIMPLANTED EARS

		Implanted Ear					*Unimplanted Ear*				
Case No.	*Preoperative Hearing*	*Segment 1*	*Segment 2*	*Segment 3*	*Segment 4*	*Total*	*Segment 1*	*Segment 2*	*Segment 3*	*Segment 4*	*Total*
2	Profound loss both ears	524	1,537	1,306	1,578	4,945	354	1,088	1,591	1,945	4,978
3	Profound loss both ears	1,283	6,507	4,605	5,023	17,418	3,076	7,144	4,059	5,041	19,320
5	Residual hearing both ears; worse on right (implanted side)	418	354	245	428	1,075	129	517	340	347	1,333
8	Profound loss both ears	921	4,250	2,939	2,781	10,891	744	3,460	3,395	2,158	9,757

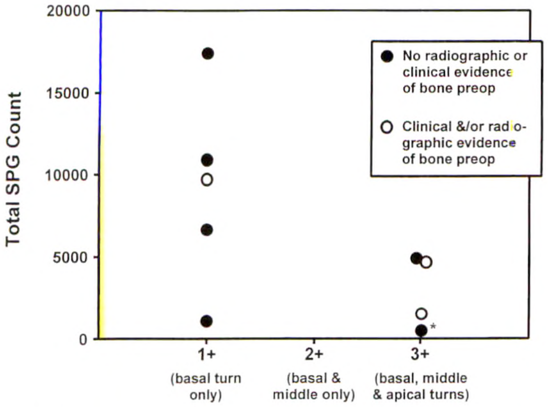

Fig 11. Scatter plot demonstrates correlation of labyrinthitis ossificans and total spiral ganglion (SPG) cell count. One case (asterisk) was from single-channel implant (case 6, right ear).

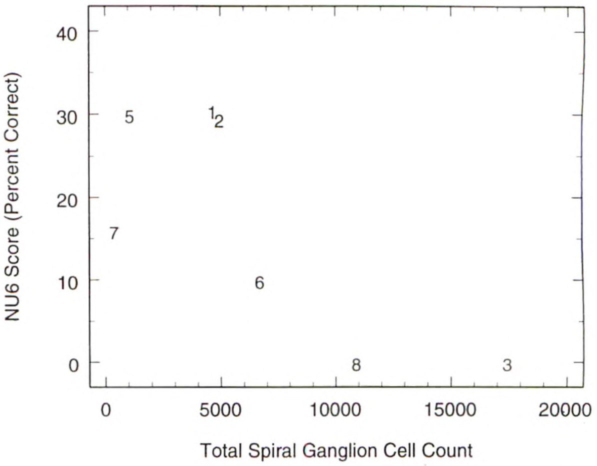

Fig 12. Implantee speech comprehension (NU-6 word score) plotted as function of total spiral ganglion cell count for each subject (see Table 3 for numerical data). Plotted numbers identify case number used to identify temporal bones.

and unimplanted sides, despite the fact that the implanted ear had worse hearing on preoperative testing.

In the fourth case (case 3), significantly fewer spiral ganglion cells were found in the 2 basal segments, and on the implanted side as compared to the nonimplanted side. This patient had a bilaterally symmetric profound hearing loss before implantation. However, in this patient, a single-channel implant placed 14 years before death was explanted 8 years before death, and then a multichannel system was implanted immediately.

As shown in Fig 11, in those temporal bones with labyrinthitis ossificans, no matter whether attributed to the preoperative period or to the postoperative period, there were fewer spiral ganglion cells in those bones with the greatest new bone formation.

Correlation of Histopathology and Speech Comprehension. Figure 12 plots NU-6 word scores as a function of the total spiral ganglion cell count for 7 subjects (see also Table 3). It is notable that the 2 individuals with the lowest number of total spiral ganglion cells (391 for case 7 and 1,075 for case 5) posted respectable NU-6 scores for the implant systems they used (16% for the Ineraid and 30% for the Nucleus 22). The best-performing subjects of this group (cases 1, 2, and 5) each had less than 15% of the average spiral ganglion cells found in temporal bones from normal-hearing individuals. The 2 individuals with the largest number of surviving spiral ganglion cells (case 3 with 17,418 and case 8 with 10,891) posted NU-6 scores under 15% (Fig 13). The apparent negative correlation between NU-6 score and total spiral ganglion cell count is unchanged when the NU-6 scores are compared with the sum of the spiral ganglion counts in segments 1 and 2 only (millimeters 0 to 15).

There was no obvious correlation between the amount of new bone within the cochlea and the results of the speech comprehension test (Fig 14). Table 4 displays the correlation of performance in speech comprehension with the scalar position of the electrode and with the total spiral ganglion cell count. In 3 cases, the electrode array traversed the plane of the basilar membrane. Although this penetration intuitively would suggest increased trauma to the or-

TABLE 3. MULTICHANNEL COCHLEAR IMPLANT NU-6 PERFORMANCE AND SPIRAL GANGLION CELL COUNTS

Case No.	*Depth of Insertion (mm)*	*Segment 1*	*Segment 2*	*Segment 3*	*Segment 4*	*Total*	*NU-6 Score (%)*
1	22	394	1,680	959	1,605	4,638	30*
2	19	524	1,537	1,306	1,578	4,945	30
3	23	1,283	6,507	4,605	5,023	17,418	0
4	22	843	4,250	2,400	2,237	9,730	N/A
5	20	418	354	245	428	1,075	30
6	22	0	3,087	1,607	1,997	6,691	10
7	21	56	102	205	28	391	16
8	24	921	4,250	2,939	2,781	10,891	13*

N/A — not available.

*Estimated (see Rabinowitz et al[27]).

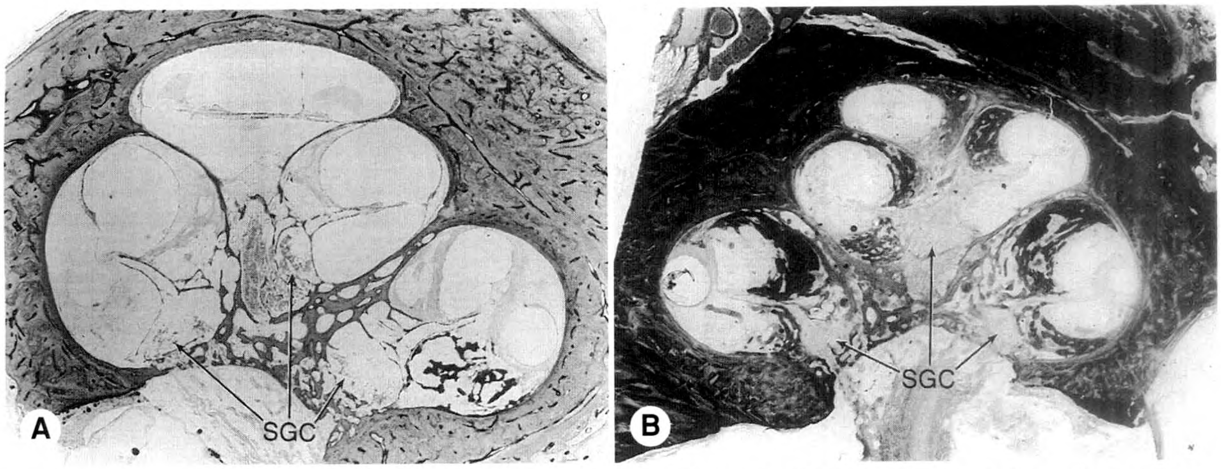

Fig 13. Midmodiolar sections. **A**) (Case 3) Residual spiral ganglion cells (SGC) can be seen in Rosenthal's canal (original ×24). Despite residual SGC count of 17,418, NU-6 word score was 0%. **B**) (Case 2) Very few SGCs can be seen in Rosenthal's canal (original ×20). Despite total SGC count of 4,945, NU-6 word score was 30%.

gan of Corti, there was no obvious impact on performance or total spiral ganglion cell count.

DISCUSSION

Immediate Trauma to Organ of Corti. In these 8 cases in which a multichannel electrode had been placed from 1 to 8 years before death, dissection of the soft tissue of the lateral cochlear wall (particularly in the ascending segment of the basal turn) was universal and consistent with data from experimental animals, human cadaveric specimens, and prior reports of in vivo implantations. In monkeys, common electrode insertion trauma has included damage to the osseous spiral lamina, basilar membrane, and spiral ligament, particularly in the basal turn.[9] Similarly, in cadaveric human specimens, tears in the spiral ligament and breaks in the basilar membrane, particularly in the basal turn, have been reported.[11-14] In specimens from patients who underwent cochlear implantation during life, dissection of the spiral ligament, fracture or dislocation of the osseous spiral lamina, and displacement or perforation of the basilar membrane have been described.[17-23] Likewise, postoperative computed tomography scans have demonstrated trans-scalar passage of electrodes.[29]

New Bone Formation. New bone formation after implantation, presumably secondary to the implantation itself, has been described both in animal specimens[1-3,9] and in human temporal bones that were implanted during life.[15,17,20,21] The formation of bone in humans after implantation does not prove that the bone formation was caused by implantation. However, the location of new bone formation in an area of universal trauma, as shown in this report, coupled with the formation of new bone in experimental animals deafened by administration of ototoxic drugs,[3] in which labyrinthitis ossificans would not be expected, or in animals whose inner ears were completely normal before implantation,[9] argues strongly that at least some new bone formation seen after implantation is caused by insertional trauma.

Effects of Insertional Trauma and New Bone Formation on Spiral Ganglion Cell Count. In the 4 pa-

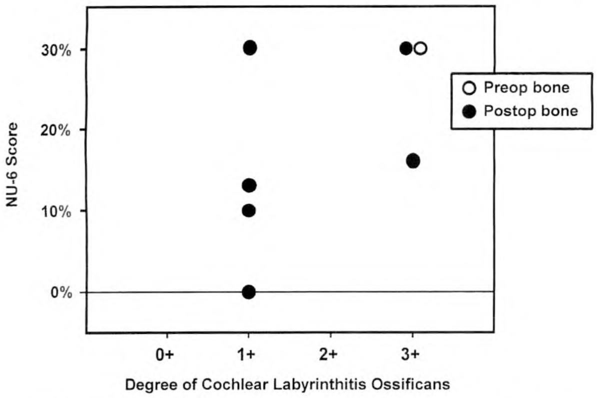

Fig 14. There was no obvious correlation between extent of labyrinthitis ossificans and speech comprehension as measured by NU-6 word score.

TABLE 4. CORRELATION OF NU-6 PERFORMANCE WITH SCALAR POSITION OF ELECTRODE (MULTICHANNEL ONLY)

Case No.	*Electrode Position*	*NU-6 Score (%)*		*Total Spiral Ganglion Cell Count*
1	SV only	30	Mean, 20	4,638
2	SV only	30		4,945
3	SV only	0		17,418
4	ST only	N/A		9,740
5	ST only	30		1,075
6	Crosses	10	Mean, 13	6,691
7	Crosses	16		391
8	Crosses	13		10,891

SV — scala vestibuli; ST — scala tympani; Crosses — crosses from ST to SV or SV to ST.

tients with multichannel implantation for whom the opposite nonimplanted temporal bone was available for comparison, there was little evidence to suggest that implantation trauma induced significant degeneration of spiral ganglion cells. Although some studies[20,22] have demonstrated an apparent decrease in the number of spiral ganglion cells on the implanted side, other studies, both in humans[17,21,23] and in experimental animals,[1-3] show no obvious decrease in spiral ganglion cell population. Furthermore, in animal studies, not only was there little evidence of degeneration of the spiral ganglion induced by implantation, but in some studies, implantation coupled with electrical stimulation resulted in preservation of spiral ganglion cells over time.[6,7]

In 1 of the 4 cases presented in this report (case 3), the spiral ganglion cell population was lower on the implanted side than on the nonimplanted side. However, this patient had had a single-channel implant explanted and a multichannel system then implanted. Thus, the decrease in spiral ganglion cell population may have been the result of increased trauma caused by the explantation and reimplantation sequence, as has been demonstrated in an experimental animal model.[9]

The negative correlation between spiral ganglion cell count and the extent of labyrinthitis ossificans as shown in this study is consistent with previous studies in humans[30] and animals.[9]

Correlation of Spiral Ganglion Cell Count With Cochlear Implant Performance During Life. Although counterintuitive, the ganglion cell data show a negative correlation between residual spiral ganglion cell counts, either basal-segment counts or total counts, and performance during life with an implant as measured by scores of single-syllable word recognition. Although animal psychophysical testing has suggested a positive correlation between low thresholds and large dynamic ranges with preservation of sensorineural structures within the inner ear,[31] in human specimens there has been little evidence to suggest correlation of implant performance as measured by speech recognition with the remaining spiral ganglion cell count.[18] However, the majority of the previously reported human cases were single-channel implantations.

Furthermore, it is also clear that factors other than auditory neural integrity are important in performance with multichannel cochlear implants. A study of 48 postlingually deafened adults[32] identified 6 preoperative measures that accounted for 61% of the intersubject variability on NU-6 word understanding. Only 2 of the 6 measures could be attributed to remaining spiral ganglion cell counts.

Although the number of temporal bones in this study is small, the negative correlation between the total spiral ganglion cell counts and single-syllable word recognition is intriguing. One interpretation of this observation is that the central nervous system is more important than peripheral neural integrity in the performance of implant users. Recent functional magnetic resonance imaging of electrically evoked brain activation patterns measured in implantees showed an abnormally small ratio of contralateral to ipsilateral excitation.[33] To the extent that these and other brain abnormalities limit implantee performance and contribute to the negative correlation between spiral ganglion cell counts and performance, the results of this study suggest that a characterization of such abnormalities and their possible effect on performance may provide insight into the design of better speech processors and electrode arrays for cochlear implants. In addition, correlation of intracochlear damage and density of spiral ganglion cells with psychophysical percepts recorded during life, as has been reported on by Kawano et al,[24] may provide valuable additional data and is planned.

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Ann Otol Rhinol Laryngol 110:2001

IMAGING CASE STUDY OF THE MONTH

ABERRANT INTERNAL CAROTID ARTERY IN THE MIDDLE EAR

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FREIBURG, GERMANY

CASE REPORT

A 33-year-old woman was referred to the Department of Otorhinolaryngology at the University Clinic Freiburg in July 2000 for evaluation of an unclear otoscopic finding. The patient reported right pulsatile tinnitus and intermittent clicking of 1 years' duration with minor hearing loss and intermittent insomnia. She had no history of vertigo, aural pressure, or otorrhea. Examination revealed a reddish pulsatile mass behind an intact and calcified tympanic membrane on the right (Fig 1).

Rinne's test was negative, and Weber's test lateralized to the right. A pure tone audiogram showed a right low-frequency conductive hearing loss of 15 dB. The left ear seemed normal. The tympanometry and stapedial reflex measurement showed a middle ear pressure curve with a course with several peaks; however, there were several small peaks synchronous with the pulse on stapedial reflex measurement. The other findings of the head and neck examination were normal.

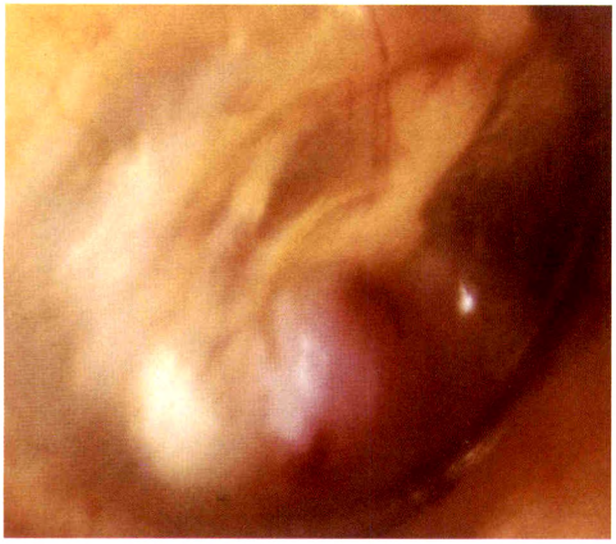

Fig 1. Right tympanic membrane with pulsatile reddish middle ear mass.

The diagnosis of a vascular tumor of the right middle ear was considered. The patient underwent high-resolution computed tomography of the temporal bones and magnetic resonance angiography (MRA). The high-resolution tomography showed an aberrant course of the right internal carotid artery (ICA), which passed through the low part of the eardrum and the middle ear in a rectangular course and was covered by a thin bone lamella (Fig 2). On MRA, a distinctly reduced caliber of the ICA with a lateral displacement on the right was seen (Fig 3). There was no evidence of a tumor or bony destruction. Furthermore, a duplication of a vein at the level of the jugular foramen was detected.

The patient did not undergo ear surgery. She was informed of the diagnosis and the danger of manipulation of the auditory canal. Regular follow-up was arranged.

DISCUSSION

Aberrant courses of petrous vessels are extremely

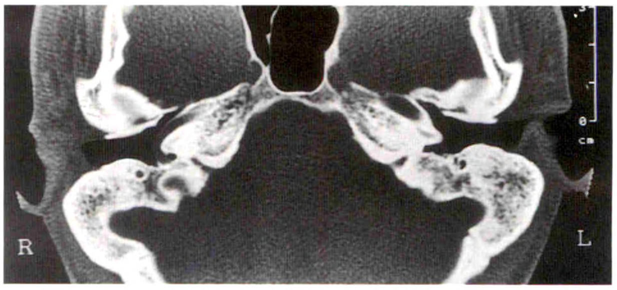

Fig 2. Axial computed tomography scan through temporal bones shows aberrant course of right internal carotid artery.

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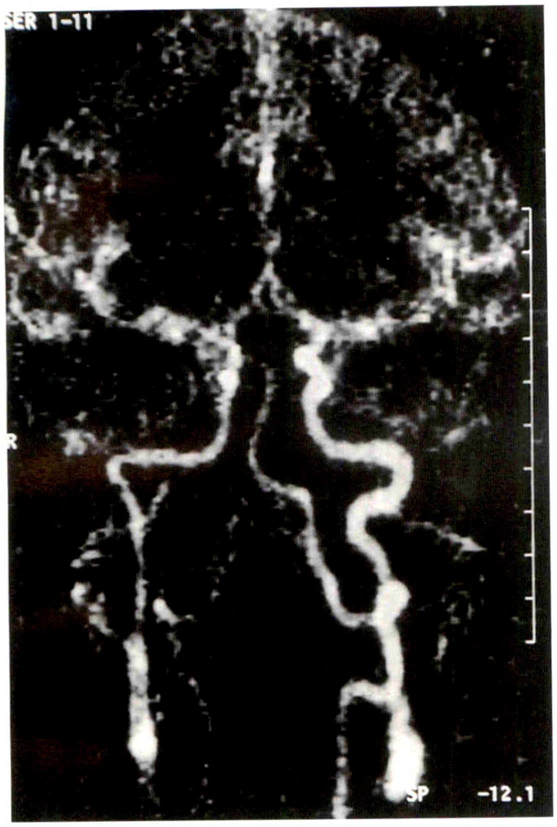

Fig 3. Magnetic resonance angiography shows lateral displacement of internal carotid artery on right and normal position on left.

rare. Only 42 clinical cases of an aberrant ICA in the middle ear had been reported until 1991 in the English-language literature.[1] In most cases, aberrant ICAs appear together with pulsatile tinnitus and hearing loss; vertigo and progressive conductive hearing loss are also described. However, the absence of symptoms does not mean that these aberrations are not present. Most cases of intratympanic ICA occur in women (5:1) and are found on the right side (2:1).

Normally, the ICA enters the petrous bone medial to the styloid process via the carotid canal, separated from the internal jugular vein by the carotid ridge. The initial vertical segment is anterior to the cochlea and separated from the tympanic cavity by a plate of bone approximately 0.5 mm thick, which may be cribriform in childhood and may be resorbed in old age.[2] The ICA then turns anteriorly to lie inferior and posteromedial to the eustachian tube, traverses the foramen lacerum, and enters the middle cranial fossa.

Anomalies of the ICA within the temporal bone include aneurysms and arterial displacement, both of which can be congenital or acquired. Several theories have been proposed to explain this anomaly.[3-5] Congenital aneurysms are thought to originate from a persistent stapedial artery[6] or abnormal persistence of embryonic vasculature that may supply enough traction to pull the ICA into the tympanic cavity. This traction may fix or pull the ICA laterally into the middle ear.[4] Another theory, supported by Lasjaunias et al,[5] involves the supposition that blood flow through a vascular channel determines the formation of vessels. Acquired aneurysms may result from head trauma, surgery, atherosclerosis, otitis media, or syphilis.[7]

Retrotympanic vascular masses are usually not specific enough to lead to diagnosis. The differential diagnosis of an aberrant carotid artery includes genuine cholesteatoma, glomus tympanicum tumor, glomus jugulare tumor, arteriovenous fistula, sinus cavernosus fistula, carotid fistula, dehiscent jugular bulb, petrous ICA aneurysm, megadolichobasilaris, persistent stapedial artery, and enlarged and posteriorly displaced middle meningeal artery.[8-11]

So far, conventional angiography has been used as the primary diagnostic imaging procedure; it shows the lateral displaced vertical segment of the ICA in the anterior-posterior view. Lapayowker et al[12] described the "vestibular line," which is a vertical line drawn tangential to the lateralmost extent of the vestibule. This line indicates the lateral limit for location of the normal intratemporal ICA. If the ICA lies lateral to this line, it is considered aberrant. These same features can be demonstrated by MRA.[8]

High-resolution tomography of the temporal bones and MRA are noninvasive imaging procedures that provide excellent visualization of the temporal bone and intracranial and extracranial vasculature without the need for contrast injection or overnight hospitalization. Today, MRA has almost superseded the need for conventional intra-arterial contrast-enhanced angiography.[8]

Myerson et al[13] described an incidence of 1% of ectopy of the ICA based on petrous bone studies. A pinpoint dehiscence of the ICA may be present in 1% of the population.[2] In rare cases, other vessel anomalies can be associated with deviant cervical or intracranial vessel divisions.[3,7]

Moreano et al[14] studied 1,000 human temporal bones and detected a carotid canal dehiscence (7.7%) and a microdehiscence (7.4%) of the carotid canal in 15.1% of all bones. Campbell et al,[15] in 1992, and Glasscock et al,[3] in 1993, each reported a case of bilateral ICAs in the middle ear.

Knowledge of this rare entity is essential to any otolaryngologist who undertakes myringotomy and ear surgery. Most authors recommend a conservative attitude toward a proven aberrant ICA. In many cases, this abnormality is asymptomatic, and it is most often diagnosed during ear surgery. However, mismanagement of this anomaly may have disastrous consequences.[16,17] A paracentesis in these cases leads to bleeding from the tympanum and eustachian tube that is usually not controllable by surgery. Therefore, preoperatively, when the symptoms are present, a vascular cause for the local findings should be considered. Henriksen et al[18] reported a case of a 7-year-old girl with aural bleeding following myringotomy. In this case, diagnostic investigations showed a congenital aberrant ICA and a pseudoaneurysm in the middle ear that was presumed to be traumatic, ie, formed after incision into the wall of the artery.

The best treatment after diagnosis of an aberrant ICA is to avoid manipulation and to inform any physician involved in the patient's care.[19,20] In a case of iatrogenic injury to an aberrant ICA during myringotomy or middle ear surgery, severe bleeding can occur.[17,21] However, in the face of chronic middle ear infection, surgical techniques to restore the middle ear cavity and to cover the aberrant vessel permanently with bone and fascia grafts are advocated by Ruggles and Reed.[22] The surgeon should be aware of the risks of consecutive hemiparesis, temporary aphasia, permanent hearing loss, and death.[4,7,20] Immediate treatment should consist of packing the external auditory canal to control the hemorrhage, even if the packing must be replaced and left in place for several weeks because of recurrent bleeding.[23] Additional steps to control hemorrhage include a posterior epistaxis pack, ICA ligation, and balloon occlusion.[20] However, interruption of blood flow in the ICA is usually not necessary. The patient should be informed of the danger of manipulation of the auditory canal. Patients should be followed up regularly.

In summary, vascular anomalies of the middle ear are fascinating problems that must be differentiated from vascular tumors.[2] We emphasize that it is important to consider the possibility of this anomaly and to perform adequate diagnostic imaging before surgical exploration.

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Ann Otol Rhinol Laryngol 110:2001

IMAGING CASE STUDY OF THE MONTH

TORNWALDT'S CYST AND MAGNETIC RESONANCE IMAGING

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INTRODUCTION

Tornwaldt's cyst is a rare benign disorder induced by persistent notocord remnants. The contact between these remnants and the pharyngeal ectoderm creates the condition for ingrowth of respiratory epithelium,[1] which forms the so-called Tornwaldt bursa.[2] This pouch drains into the nasopharyngeal cavity and develops into a cyst only in cases of partial or total obstruction of the orifice as a result of infection or after adenoidectomy. The aim of this report is to present a case of Tornwaldt's cyst and point out the importance of magnetic resonance imaging (MRI) in its diagnosis.

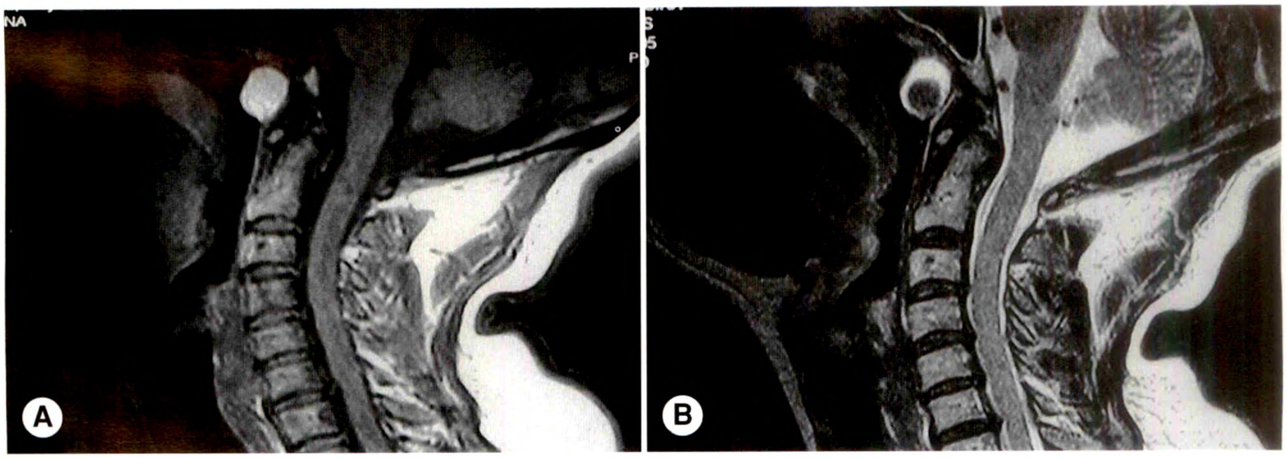
CASE REPORT

A 54-year-old woman came to our department complaining of halitosis and occasional purulent and/or mucous discharge from the rhinopharynx. The otolaryngological examination revealed that the patient had an approximately 2-cm mass on the midline in the posterior-superior angle of the nasopharynx. It appeared to be a cyst and had a small orifice (typical of Tornwaldt's cyst). A computed tomography (CT) scan of the nasopharyngeal space confirmed the presence of a solid mass with well-defined borders. T1-weighted and T2-weighted MRI produced hyperintense images that clearly revealed the mass's cystic nature (see Figure). A diagnosis of Tornwaldt's cyst was made.

Endoscopic surgical removal with 30° and 70° rigid fiberoptic endoscopes was planned. Access to the nasopharyngeal area was obtained by retracting the uvula. This technique allowed us to remove the cyst and its fistula under constant visual control of the specimen, thus avoiding undesired trauma to the tissues and reducing the risk of recurrence. The patient was discharged 48 hours later with antibiotic therapy. Histologic examination confirmed the diagnosis of a cystic mass covered with respiratory epithelium with stroma below, containing little lymphoid tissue and mixed glands. The cyst and its fistula had been totally eradicated. The patient was reassessed at 15, 30, and 90 days after operation. The fiberoptic view demonstrated excellent wound healing.

DISCUSSION

The incidence of Tornwaldt's cyst is 4% on autopsy, with a predilection for clinical presentation in



Sagittal magnetic resonance images of nasopharynx. **A**) T1-weighted image. Cyst appears as solid mass. **B**) T2-weighted image. Fluid component in cyst is evident.

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IMAGING CHARACTERISTICS OF VARIOUS NASOPHARYNGEAL DISORDERS

Disorder	*Computed Tomography*	*Magnetic Resonance Imaging*		*Site*
		T1-weighted	*T2-weighted*	
Adenoid retention cyst	Solid tissue	Isointense	Hyperintense	Posterior
Meningocele	Bony defect	Isointense	Hyperintense	Superior
Meningoencephalocele	Bony defect	Isointense	Hyperintense	Superior
Sphenoid sinus mucocele	Bony defect	Isointense	Hyperintense	Superior
Rathke's pouch cyst	Solid tissue	Isointense	Hyperintense	Superior
Branchial cleft cyst	Solid tissue	Hyperintense	Hyperintense	Posterior-lateral
Tornwaldt's cyst	Solid tissue	Hyperintense	Hyperintense	Posterior

the second and third decades of life. Magnetic resonance imaging not necessarily performed for nasopharyngeal disorders showed slightly different rates, varying from 0.2% to 5%, and the peak incidence was seen at 40 to 60 years.[3-5] It may be that the symptoms appear gradually later on in life, forcing the patient to have a checkup and undergo diagnostic tests.

In fact, Tornwaldt's cyst is usually asymptomatic. The first symptoms may start to appear after spontaneous drainage into the nasopharyngeal cavity and as a result of the involvement of the nervous plexus in this site, which can induce occipital headaches, cervical myalgia, halitosis, and middle ear effusion.

Clinical diagnosis is easily made with flexible or rigid endoscopy. The cyst appears as a single midline mass on the posterior wall of the nasopharynx, from a few millimeters to 1 or 2 cm in size, with a smooth surface through which the liquid contents can be seen. A small orifice through which the cyst drains periodically may be visible nearby.

In the differential diagnosis from other typical rhinopharyngeal disorders (such as Rathke's pouch, adenoid retention cyst, meningocele, meningoencephalocele, branchial cleft cyst), it is mandatory to combine endoscopic examination with imaging techniques (CT, MRI) and histologic examination. To be more specific, Tornwaldt's cyst has a solid aspect on CT, whereas it is hyperintense on T1- and T2-weighted MRI. Neither CT with contrast medium nor MRI with gadolinium provides any further details.

To facilitate the differential diagnosis, the Table summarizes the characteristics of the various nasopharyngeal disorders as they appear on different imaging techniques.

Rathke's pouch is usually situated in the upper portion of the nasopharynx and has a solid aspect on a CT scan. Adenoid retention cysts are small (<5 mm in diameter), multiple masses that appear on CT as solid lesions and are seen to be typical lymphoid tissue on histologic examination.[6] Meningoceles and meningoencephaloceles are all to be found on the vault of the nasopharynx and show bony defects on CT. Mucoceles of the sphenoid sinus have a location and CT appearance similar to those of these latter disorders, but they are differentiated by involvement of the sinus floor. All of these disorders (Rathke's pouch cyst, adenoid retention cyst, meningocele, meningoencephalocele, branchial cleft cyst, Tornwaldt's cyst) are hyperintense on T2-weighted images, and apart from Tornwaldt's cyst and branchial cleft cyst, which appear hyperintense on T1-weighted images, all are isointense on T1-weighted images.

The differential diagnosis between Tornwaldt's cyst and branchial cleft cyst is in the location. A branchial cleft cyst usually lies more laterally, close to the eustachian tube orifice. Its CT appearance is solid.[2,3,5]

Tornwaldt's cysts are treated surgically.[7] The indication depends strictly upon the appearance of symptoms. The use of nasal endoscopy has considerably facilitated the cysts' surgical removal, because of its capacity to provide precise visual control during the operative procedure.

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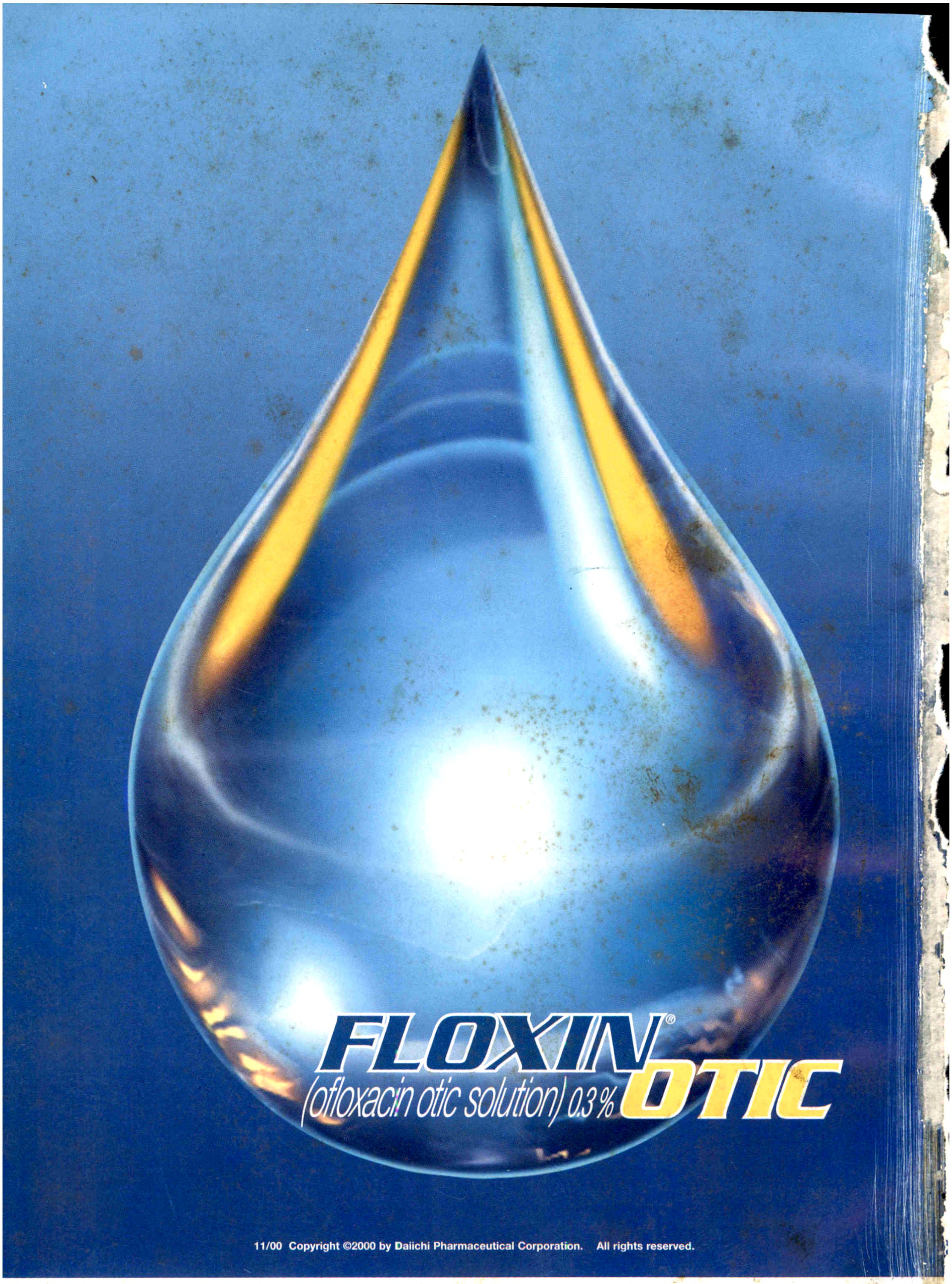

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